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FABRICATION AND CHARACTERISATION OF POLYMER COMPOSITES FOR ENDODONTIC APPLICATIONS

**A thesis submitted for the degree of
Doctor of Philosophy**

By

Raghad Abdulrazzaq Al-Hashimi

**Dental Institute
King's College London
London, United Kingdom**

2014

Abstract

The success of root canal treatment is dependent on canal debridement, disinfection and impervious obturation of the root canal system. The material most commonly used for root canal obturation is the *trans*-isomer of isoprene, known as gutta-percha. Limitations of gutta-percha as a root canal obturation material include lack of flexibility and potential for degradation caused by eugenol or other components of root canal sealers and restorative materials.

A detailed study on the development, characterisation and *in vitro* evaluation of a composite, tailor-made to function as a root canal obturation material as well as serve as a “carrier” for delivering a root canal obturation material was undertaken. Polymer composites comprising of low density polyethylene (LDPE), hydroxyapatite (HA) or 45S5 Bioglass® (BAG) and strontium oxide (SrO) were developed via extrusion using a single screw extruder and drawn into the desired shape using appropriate ‘dies’ and optimised based on their physical and mechanical properties.

The polyethylene in the composite showed no evidence of degradation post extrusion as confirmed by FTIR spectroscopy, the thermal analysis showed that the HA/PE composites had melting point of 110.5-111.2°C, much higher than that of gutta-percha (52°C). The tensile strength and elastic modulus of the silanated HA/PE composites were significantly higher than those of gutta-percha ($p < 0.0001$) under dry conditions and after storage in simulated body fluid. Furthermore, the interaction of eugenol did not produce any change in the experimental HA/PE composites whereas gutta-percha showed a significant increase in the weight. The radiological contrast of the silanated HA/PE fibres was adequate to allow their use as a root canal obturation material. Bioglass® reinforced low density polyethylene with SrO composites are promising obturation materials for endodontic treatment. They exhibited superior stiffness in comparison to gutta-percha with excellent biocompatibility and bioactivity. An enhanced proliferation of human osteoblast cells was also observed.

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List of abbreviations

PLLA:	Poly-L lactic acid
PHB:	Polyhydroxybutrate
LDPE:	Low density polyethylene
HA:	Hydroxyapatite
HCA	Hydroxy carbonate apatite
BAG:	Bioactive glass
SrO	Strontium oxide
GC:	GuttaCore
GP:	Gutta-percha
MTA:	Mineral trioxide aggregate
HOB:	Human osteoblast
SBF:	Simulated body fluid
DSC:	Differential scanning calorimetry
DMA:	Dynamic mechanical analysis
FTIR:	Fourier transform infrared spectroscopy
SEM:	Scanning electron microscopy
μ CT:	Micro-Computed Tomography
MTT:	Methyltetrazolium
TMX:	Thermanox TM
MMP:	Matrix metalloproteinase
μ A:	Microampere
Pa:	Pascal
GPa:	Giga pascal
MPa:	Mega pascal
SD:	Standard deviation

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Introduction

Obturation of the root canal system is essential in preventing apical periodontitis progression and in promoting apical healing. This can be accomplished by using an appropriate root canal obturation material which reduces microleakage and entombs residual bacteria within the root canal space. Ideally, a material used for root canal obturation must have some requirements enabling it to fill the entire root canal system. Currently, a plethora of obturation methods are used, ranging from lateral compaction to a variety of thermo-plasticised gutta-percha techniques.

Many different materials based on polymer technology have been advocated as root canal obturation materials; however with the prevailing standards of obturating root canals with gutta-percha and sealers, development of newer materials with properties better suited for this application was limited. There are a multitude of considerations which must be taken into account when planning to develop new root canal obturation materials. Ideally, a material used for root canal obturation must be biocompatible, non-resorbable, impervious to dissolution by tissue fluids, provide a hermetic seal, sterilisable, easily retrieved on demand and must possess good handling characteristics. A modulus of elasticity approaching that of dentine might help prevent root fractures (Hiraishi *et al.* 2005, Williams *et al.* 2006). Although gutta-percha is widely used for root canal obturation due to its thermoplasticity and simplicity, it still has limitations (de Souza Filho *et al.* 2012), including the inability to strengthen the endodontically treated tooth (Teixeira *et al.* 2004) and the impossibility to be sterilised by heat application (Prado *et al.* 2011). In addition, the sealing ability of gutta-percha is

clinically unsatisfactory as it does not adhere to the tooth structure (Shipper & Trope 2004).

Various root canal obturation materials based on polymer composites such as Resilon[™] (polycaprolactone-based composite), polyurethane-based composites and polycaprolactone phospho-glass based composites were introduced in the endodontic field. Potentially, a root obturation material should be sufficiently stiff to conform to the irregularities of the root canal space, providing both an apical and coronal seal. In the meantime, the stiffness of the composite materials developed as a new root canal obturation material could help reinforce endodontically treated roots and prevent their fractures. Thus, in the present study low density polyethylene hydroxyapatite composites were tailored to function as a main obturation core material. Composite fibres of low density polyethylene Bioglass[®] were also fabricated in this study with the aim of developing bioactivity and an antibacterial effect. Various analytical tests were conducted to assess the suitability of HA or BAG/PE materials for endodontic use as obturation core materials.

Carrier-based root canal obturation is a popular and an effective method to create a three-dimensional obturation. It consists of a flexible plastic or gutta-percha carrier coated with a layer of root obturation material such as gutta-percha or Resilon[™]. However, concerns were reported by clinicians in terms of inability to seal oval-shaped root canals, lack of apical control and in particular, concerning the difficulty associated with the removal of the carrier during endodontic re-treatments. Indeed, the development of new carrier-based obturators would be advantageous to facilitate the retreatment procedures in case of failure of the endodontic treatment. In the present study, specifically designed carrier materials for delivering gutta-percha into the root canal space as a core carrier for carrier-

based root canal obturation were developed and characterised. The carrier material is made up of polyethylene and hydroxyapatite mixed with strontium oxide. The concentrations of LDPE/HA with SrO were determined and optimised for clinical handlings as prime consideration. Figure i.0.1 summarizes the workflow of our research.

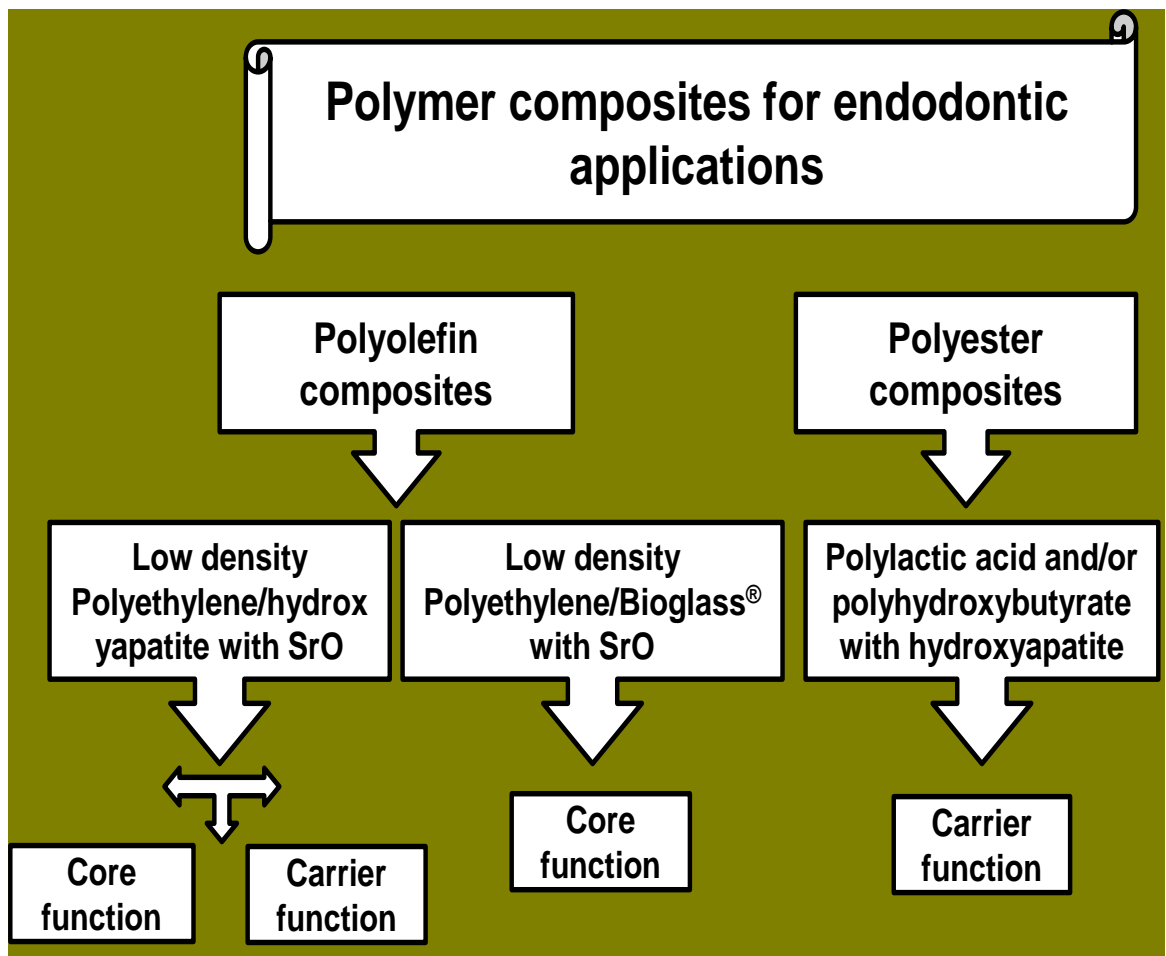


Figure i.0.1: Flowchart representing composites fabricated in this study for endodontic applications

Research aims

1. Development and characterisation of polymer-based composites with properties tailor-made to function as a root canal obturation material using a melt-extrusion process.
2. Development and characterisation of polymer-based composites to function as “carrier” for carrier-based root canal obturation using a melt extrusion process.
3. Compare and characterise the affinity between the carrier materials developed in the present study and the gutta-percha coating around the carrier material in carrier-based root canal obturation systems.
4. Qualitative and quantitative assessment of specifically designed carrier-based root canal obturation systems using physical and *in vitro* test methods.

Chapter 1 Literature review

1.1 Non-surgical endodontic treatment

Non-surgical root canal treatment is a predictable treatment of the inflamed or infected root canal space accomplished by chemo-mechanical debridement and obturation of the root canal space with an appropriate root canal obturation material (European Society of Endodontology Guidelines 2006, Gutmann *et al.* 2006, Ng *et al.* 2008, Ng *et al.* 2011, Paredes-Vieyra & Enriquez 2012). The overall goal of the root canal treatment is to prevent and/or treat apical periodontitis (Ørstavik & Pitt Ford 2008), promoting recovery of the infected periradicular tissue (Ørstavik 1996, Gillen *et al.* 2011). Apical periodontitis is an inflammatory reaction of the periradicular tissues as well as an immunological response of the host defence system to microbes that have infected the root canal space (Torabinejad *et al.* 1985). The root canal space is generally invaded and then infected through different ways including caries, defective restorations, dentine cracks, traumatic injuries, tooth wear, and periodontal disease (Siqueira Jr & Rocas 2011).

The success rate of non-surgical root canal treatment currently stands at around 85.9%-94% (Imura *et al.* 2007). The outcome of root canal treatment can be determined by several challenging issues. These issues are prognostic in nature based on using clinical and/or radiographic signs of periapical healing as an outcome measure (Friedman *et al.* 2003). Although apical periodontitis is the main prognostic factor in initial endodontic treatment, other studies have considered tooth survival as an outcome measure of root canal treatment (Caplan *et al.* 2002, Ng *et al.* 2010). The success rate of endodontically treated

teeth without periapical radiolucencies is generally higher than that of teeth with radiolucencies (Molven & Halse 1988).

Components of root canal system

The irregularities and curvatures of the root canal space as shown in Figure 1.1 necessitate considerable effort from the clinician whilst planning to undertake a root canal treatment. Thus, careful understanding of the morphology of the root canal space may help increase the success rate of root canal treatment (Vertucci 2005). The dentine constituents, collagen-rich organic matrix reinforced by calcium phosphate mineral particles with water, are ably optimised to different mechanical demands in the mouth. The distribution of mineral phase provides the elastic modulus of dentine (stiffness) while the toughening mechanisms are assigned to collagen fibres and water content (Kishen 2006).

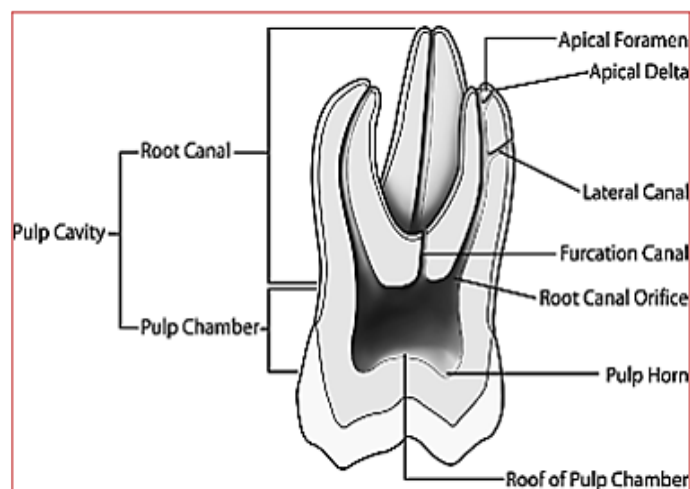


Figure 1.1: Components of the root canal system

1.2 Factors affecting the outcome of root canal treatment

A number of potential factors have been reported that may influence the success of endodontic treatment, which include, quality of the root obturation material (Ng *et al.* 2011), persistence or progression of apical periodontitis, age, tooth type, preoperative periapical status, occlusion, type of final restoration and the quality of tooth/restoration margin (Lee *et al.* 2012). Beside these factors, recent studies indicated that homogeneity and apical extension of root canal obturation have a significant effect on the periapical healing but not on the survival of the treated teeth (Ng *et al.* 2008, Ng *et al.* 2010, Lee *et al.* 2012). Much effort has been made to investigate the factors affecting the outcome of primary and secondary root canal treatment (Caplan *et al.* 2002, Ng *et al.* 2011). Tooth survival has been used as an outcome measure in these controlled studies. Increased tooth survival was found in non-diabetic patients teeth with absence of pre-operative deep periodontal probing defects, pain and sinus tract; absence of pre- and intra-operative tooth perforation; achievement of patency at canal terminus; absence of root filling extrusion; teeth with cast restoration after treatment; teeth with both mesial and distal adjacent teeth present; and teeth not requiring cast post and core for support and retention of restoration (Ng *et al.* 2011).

1.3 Clinical stages of non-surgical endodontic treatment

1.3.1 Cleaning and shaping procedure and its effect on the success of endodontic therapy

The prime objective of root canal preparation is to eliminate the infected pulp tissue, microorganisms and their by-products from root canal system whilst establishing a proper canal shape to receiving an appropriate root canal obturation material (Schilder 1974, Siqueira *et al.* 1997). Biofilms and their by-products can be removed by using a combination of mechanical instrumentation

and irrigating solutions in conjunction with application of intracanal medicaments between appointments (Sjogren *et al.* 1997, Paredes-Vieyra & Enriquez 2012). Clinical criteria for a good root canal shaping include (Pettiette *et al.* 1999).

- Continuously tapered preparation
- Maintenance of original anatomy
- Position of the apical foramen maintained
- Foramen diameter as small as practical

Shaping the root canal by establishing a continuously tapering funnel shape is important to allow the removal of bacteria and their by-products from the root canal system (Sjogren *et al.* 1997). It also helps providing the perfect shape for three-dimensional obturation with gutta-percha (West 2008).

Although a vast number of root canal preparation techniques consisting of a range of hand and/or mechanical instruments are available, it still remains impossible to clean and shape the root canal system thoroughly due to presence of fins, isthmus irregularities and cul-de-sacs in the root canal space (Shuping *et al.* 2000, Gutarts *et al.* 2005). Therefore, untouched root canal surfaces may remain, leaving some residual bacterial entities in the root canal space that might jeopardise and complicate the treatment outcome (Bystrom & Sundqvist 1981). This leads to the belief that utilising effective root canal irrigation is essential for eliminating residual microbes, their by-products, and pulp debris from root canal space creating a favourable biologic environment for the healing process.

It is also necessary to consider the effects of root canal treatment procedures on the mechanical properties of the remaining tooth structure. Instrumentation, irrigation, medication, and obturation during endodontic treatment procedures

significantly affect the endodontically treated tooth in terms of (Mannocci *et al.* 2008):

- Loss of tooth structure
- Dehydration of dentine
- Dentinal collagen structure effects
- Effect of root canal irrigation and medications
- Loss of proprioception

As a result of these cumulative effects, endodontically treated teeth become more susceptible to fracture than teeth with vital pulp.

1.3.1.1 Loss of tooth structure

Root canal preparation may weaken the root and diminish the ability of the residual tooth structure to resist occlusal forces (Trope *et al.* 1985, Zandbiglari *et al.* 2006). An *in vitro* study was carried out by Wu *et al.* (2004) comparing the force required to vertically fracture non-instrumented and instrumented lower premolars with round and oval canals and canines. It was concluded that non-instrumented lower premolars exhibited a superior fracture resistance to that of instrumented teeth as the force required to fracture the instrumented premolars and canines was 30% and 2% lower, respectively than that required to fracture their non-instrumented counterparts. No significant difference in resistance to vertical root fracture was found between the round- and oval-canal premolars with and without instrumentation.

The quantity of hard tooth tissue structure lost depends on the level of caries removal, access cavity preparation, root canal instrumentation and subsequent restorative procedures. A recent study attempted to measure the volume of hard tooth tissue lost at each stage of root canal treatment procedure after caries

removal, access cavity preparation, root canal preparation, fibre post space and cast post preparation in carious premolar teeth, using micro-computed tomography. Both access cavity and post space preparation generated a significant loss of hard tooth tissue and fibre post space preparation caused a lesser loss of tooth structure than cast post space preparation (Ikram *et al.* 2009). An earlier study showed supporting observations wherein extracted upper central incisor teeth underwent access cavity preparation, manual instrumentation and tapered and parallel-sided post preparation. A significant reduction in fracture resistance (destabilization) of treated teeth after both access preparation and post preparation was demonstrated (Lang *et al.* 2006).

1.3.1.2 Dehydration of dentine

Dentine, in a healthy tooth is a porous hydrated composite tissue which forms the main part of human teeth (Kishen & Asundi 2005). However, in non-vital teeth the dentine structure is exposed to dehydration, which has been noted to cause a significant decrease in the toughness of dentine of pulpless teeth (Arola & Reprogl 2005), wherein they investigated the influence of age on the mechanical behaviour of dentine using four-point bending and fatigue tests. They showed that the rate of damage in dentine with old dentine specimens (ageing specimens) was significantly increased. An earlier study was designed by Jameson *et al.* (1993) investigating the impact of dehydration and rehydration on the brittleness and toughness of human dentine using three-point bending and tensile tests, wherein a brittle behaviour of dehydrated dentine and decreased strain at fracture was reported. A contrary study demonstrated that the dehydration of human dentine would increase the elastic modulus and ultimate tensile strength (Huang *et al.* 1992). They also demonstrated no significant differences in the compressive and tensile strengths between dentine from

devitalised and vital teeth. However, these findings need careful interpretation due to the results being obtained in an *in vitro* environment.

1.3.1.3 Effect of canal preparation on dentinal collagen structure

The organic matrix of dentine is composed of predominantly Type I collagen fibrils (Marshall *et al.* 1997) embedded in an amorphous apatite. The cross-linking of these collagen fibrils helps to impart the mechanical properties of dentine such as rigidity, stretch-resistance and high tensile strength. One of the consequences of root canal preparation is the alteration of collagen. A previous study showed that biofilm toxins and a host of-derived matrix metalloproteases (MMPs¹) may contribute to the degradation of collagen fibrils following endodontic treatment, producing brittleness of root treated teeth (Ferrari *et al.* 2004). Much effort has been made to attribute the collagenolytic activity to the potential release of MMPs during demineralization under pathological (bacterial acid production) or environmental (zinc phosphate post cementation) conditions (Tjaderhane *et al.* 1998). MMPs activated by bacterial acids also play a significant role in the destruction of dentine. However, other investigators demonstrated that collagen degradation can also occur during aging by a slow release a host of-derived MMPs over time (Pashley *et al.* 2004).

1.3.1.4 Effect of root canal irrigants

Root canal irrigants play a pivotal role in the success of endodontic treatment accomplished with either hand and/or instrumentation techniques by removing the inflamed and necrotic tissue, microbes/biofilms from the root canal space (Haapasalo *et al.* 2010). Cleaning and shaping of the root-canal space results in the formation of a smear layer, consisting of dentine particles embedded in an

¹ MMPs are a family of zinc-dependent proteolytic enzymes that have the ability to degrade the dentine organic matrix after demineralization

amorphous mass of organic material that covers the instrumented area of the root canal walls (Shen & Haapasalo 2008). Removal of the acquired smear layer necessitates the use of both a chelating or other demineralizing agent and a soft-tissue solvent because no single solution has the ability to provide both effects (Zehnder *et al.* 2005). It is believed that eliminating the smear layer helps in dissolving attached bacteria and their by-products from the root canal walls and improve the seal of root canal obturation (Torabinejad *et al.* 2002). Structural changes of dentine substrate may be generated with the use of root canal irrigation, resulting in a significant drop in elastic modulus or flexural strength values of sound root dentine, in particular following exposure to sodium hypochlorite concentrations of 5% and above (Eldeniz *et al.* 2005)

1.3.1.5 Effect of intracanal medicaments

Several substances such as calcium hydroxide (CaOH_2) (Trope *et al.* 1995b) and Ledermix (Chen *et al.* 2008) have been used as intra-canal medications in between root canal treatment appointments. Calcium hydroxide is widely recommended as an intracanal dressing to help control infection. In addition, it also activates tissue enzymes like alkaline phosphatase, producing a mineralizing effect (Estrela *et al.* 1995). The main biomechanical consequence of the prolonged use of calcium hydroxide on dentine of pulpless teeth is potential weakening of the dental structure making the tooth more susceptible to fracture (Cvek 1992, Andreasen *et al.* 2002). Owing to the high alkalinity of calcium hydroxide, alterations in the dentine substrate is expected to take place that may lead to a significant decrease in the flexural strength of dentine (Grigoratos *et al.* 2001) and eventually root fracture (Andreasen *et al.* 2002). As a result of these undesirable consequences which may cause weakening of the root, it would be prudent to consider reinforcement of the residual tooth structure whilst obturating

the prepared root canal space by selecting an appropriate root canal obturation material.

1.3.1.6 Loss of proprioception

Owing to loss of proprioception within the pulp tissue, an increased loading threshold in root filled teeth would be expected. This may result in an undesirable effects (i.e. fracture) on the remaining tooth structure (Loewenstein & Rathkamp 1955).

1.3.2 Primary functions of root canal obturation materials

The three important functions associated with a root canal obturation material are (Sundqvist & Figdor, 1998):

1. Stop coronal leakage by providing a seal against bacterial ingrowth from the oral cavity.
2. Entomb surviving microorganisms and their by-products.
3. Prevent accumulation of stagnant fluid, as shown in Figure 1.2

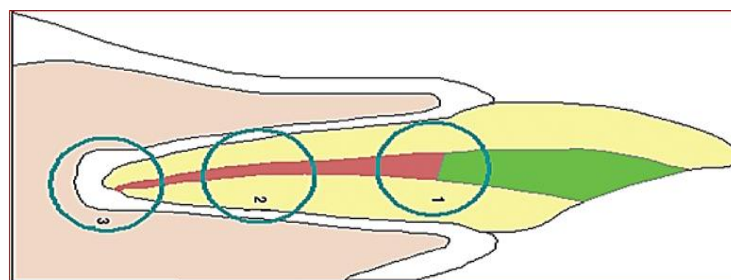


Figure 1.2: Primary function of a root canal obturation. 1, stop coronal leakage; 2, entomb surviving microorganisms; 3, prevent accumulation of stagnant fluid (adapted from Sundqvist & Fidgor 1998)

1.3.3 Requirements of an ideal root canal obturation material

The properties of an ideal root canal obturation material as outlined by Grossman (1988) include:

- Easy manipulation with ample working time
- Radiopacity and discernibility in radiographs
- Dimensional stability with no shrinkage following insertion
- Ability to seal the canal laterally and apically
- Lack of porosities
- Inhibition of bacterial growth
- Inability to produce irritation of the periapical tissues
- Lack of corrosion or oxidization
- Inability to discolour the tooth structure
- Easy removal from the root canal
- Sterility

1.3.4 Core materials currently utilised for root canal obturation

A variety of materials were reported for endodontic use over two centuries, particularly to fill the canal space, namely amalgam, asbestos, balsam, bamboo, copper, gold foil, iron, lead, oxy-chloride of zinc, paraffin, plaster of Paris, tin foil. However, none of these materials meets the requirements of an ideal root canal obturation material. An interesting review described the ability of new root obturation materials to improve the seal of the root canal system and attempted to classify obturation materials into four categories apart from gutta-percha according to their sealing ability (Li *et al.* 2014b):

- Materials that adhere to the radicular dentine and root obturation materials to eliminate the interfacial gaps

- Materials that aim to self-seal gaps by setting or hygroscopic expansion
- Materials that enhance flow and adaptation of the obturation material to canal wall
- Bioactive materials

1.3.4.1 ***Gutta-percha and its limitations as a root canal obturation material***

Gutta-percha is the most commonly used root canal obturation material and considered to be a “gold standard” in comparison to other obturation materials (Walton & Torabinejad 2002). Gutta-percha is derived from the dried juice of the Baine tree (*isonandra percha*) which is common in South East Asia, particularly in the Malaysian and Indonesian archipelago (Spangberg 1982). Gutta-percha is the *trans*- isomer of polyisoprene; its chemical structure is 1, 4, *trans*-polyisoprene as shown in Figure 1.3.

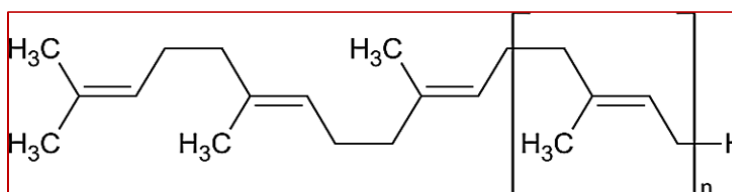


Figure 1.3: Chemical structure of gutta-percha (*trans*-polyisoprene)

Gutta-percha exists in two crystalline forms, alpha & beta, which are derived from the tree. The alpha phase of gutta-percha is obtained directly from the tree without conducting any chemical processing, whereas several chemical steps are performed to prepare the beta phase (Goodman *et al.* 1974). When the beta form of gutta-percha is thermally characterised, two endothermic peaks can be observed using differential scanning calorimetry (DSC). The first peak appears approximately at 42-49°C and represents the transformation from the beta phase

to the alpha phase, whereas the second peak appears around 53-59°C, and is attributed to the transition of the alpha material to the amorphous state of gutta-percha (Schilder *et al.* 1974). Thus beta phase gutta-percha is a solid mass that is compactible and can undergo elongation. Once it is heated gutta-percha changes to the alpha phase and becomes pliable and tacky. It can then be adapted to flow when pressure is applied (Combe *et al.* 2001). Therefore, alpha phase gutta-percha is used successfully in thermoplastic obturation techniques. Gutta-percha points normally include approximately 19-20% gutta-percha as a *trans*-polyisoprene polymer, in combination with about 59-78% zinc oxide (ZnO), with small percentages of waxes, colouring agents, antioxidants and metallic salts (Gutmann & Witherspoon 2002).

The most common limitations of gutta-percha are:

- Inability to prevent leakage (Swanson & Madison 1987, Torabinejad *et al.* 1990)
- Lack of rigidity and ease of displacement under pressure, particularly for smaller point sizes (Gesi *et al.* 2005, Johnson & Kulild 2010).
- Lack of adhesiveness as it does not bond to tooth structure and root canal sealers (Lee *et al.* 2002a).
- Gutta-percha does not strengthen endodontically treated teeth (Teixeira *et al.* 2004) .
- Complete removal of gutta-percha is challenging during retreatment or post space preparation (Ferreira *et al.* 2001).
- Gutta-percha shrinks during cooling, approximately 5-7% (Lee *et al.* 1997, Meyer *et al.* 2006).

- Physicochemical instability by the interaction with eugenol containing products (Tagger *et al.* 2003, Chandrasekhar *et al.* 2011)
- Inability to sterilize it in autoclaves or use other conventional sterilization techniques (Stabholz *et al.* 1987).
- *Trans*-polyisoprene degradation by application of high temperature particularly in thermoplasticised gutta-percha obturation techniques (Maniglia-Ferreira *et al.* 2008).
- Microbial degradation of gutta-percha by a rare microbe, namely *Nocardia nova SH22a* (Warneke *et al.* 2007, Luo *et al.* 2013). It is a bacterium capable of degrading gutta-percha and natural rubber.
- Aged gutta-percha is brittle (Maniglia-Ferreira *et al.* 2007).
- Scatter caused by gutta-percha root canal obturation material may cause artefacts in Cone Beam Computed Tomography images (Patel *et al.* 2013).

1.3.4.2 *Resilon™ and its limitations in root canal obturation*

Lack of gutta-percha adherence to the root canal walls has led to the development of new adhesive root canal obturation materials such as Resilon™ (Pentron Clinical Technologies, Wallingford, CT, USA), to create a double bond, one between a central core material and the root canal sealer and a second one between the sealer material and the root canal walls. Resilon™, a thermoplastic synthetic polyester-based root canal obturation material, was introduced in 2004 (Shipper *et al.* 2004), and consists of three major components .:

- Resilon™ core material is made up of thermoplastic polyester (polycaprolactone) as a matrix, bioactive glass, bismuth oxychloride, and barium sulphate as fillers. The filler content is approximately 65% by weight.
- Resin sealer (Epiphany/RealSeal) is a dual cure resin-based composite sealer. The resin matrix is composed of a bisphenol- A-glycidylmethacrylate (BisGMA), ethoxylated BisGMA, urethanedimethacrylate (UDMA) and hydrophilic dimethacrylates. It contains fillers such as calcium hydroxide, barium sulphate, barium glass, and silica.
- The primer is a self-etching dentine bonding agent which contains sulphonic acid-terminated functional monomer, 2-hydroxyethylmethacrylate (HEMA), water, and a polymerisation initiator.

The handling and manipulation properties of Resilon™ are similar to those of gutta-percha, and therefore the material can be used with traditional obturation techniques. As mentioned earlier, the benefit of the Resilon™ system is to allow for the adhesion of the obturation material, sealer and dentine to one another with the aim of creating a “monoblock”. Monoblocks may be classified as primary, secondary and tertiary depending upon the number of interfaces present between

the bonding substrate and the central core obturation material (Tay & Pashley 2007), as shown in Figure 1.4

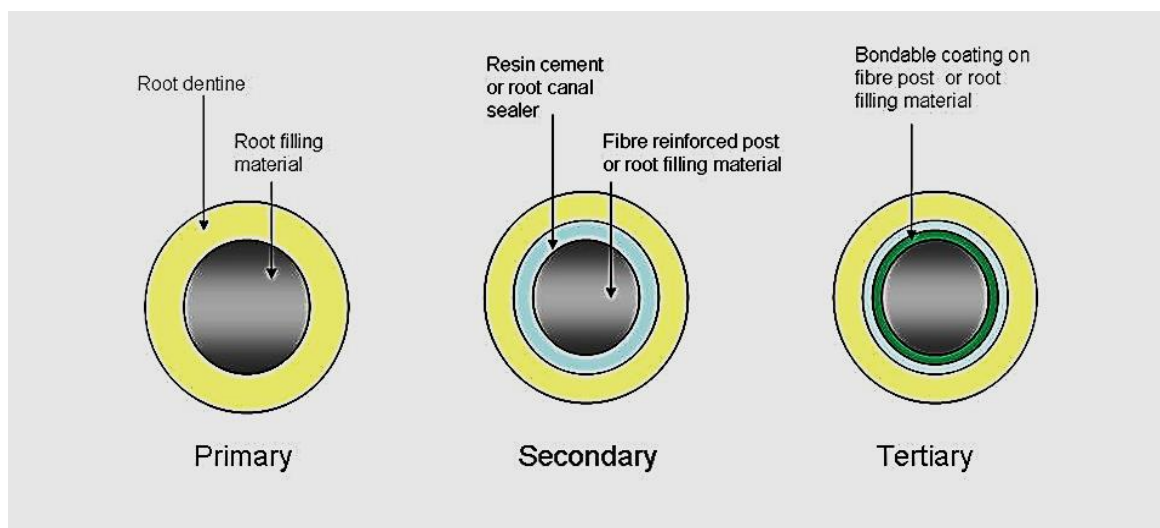


Figure 1.4: Cross sectional illustration demonstrating the classifications of endodontic monoblock, (adapted from Tay & Pashley 2007).

Over one decade ago a series of *in vitro* and *ex vivo* studies were conducted to compare the sealing ability of Resilon™ and gutta-percha. Several studies demonstrated that Resilon™ was superior to gutta-percha in achieving both apical and coronal seal and offered relevant benefits over gutta-percha as shown in Table 1.1. Other studies reported no significant differences between gutta-percha and Resilon™ in preventing apical and coronal leakage as shown in Table 1.2, while some studies showed the superiority of gutta-percha to Resilon™ in preventing apical and coronal leakage as shown in Table 1.3. In addition, the clinical reality of a “monoblock” within the root canal system is questionable because of the mechanical and anatomical challenges of bonding to the root canal walls (Tay & Pashley 2007).

Table 1.1: Studies demonstrating superiority of Resilon™ to gutta-percha in preventing apical and coronal leakage

Reference	Type of study	Apical or coronal seal tested	Leakage model	Duration	Sample size
(Shipper <i>et al.</i> 2004)	<i>In vitro</i>	Coronal	Bacterial	30 days	156
(Shipper <i>et al.</i> 2005)	Animal (dog)	Coronal	Bacterial	14 weeks	56
(Aptekar & Ginnan 2006)	<i>In vitro</i>	Apical	Dye	3 months	66
(Stratton <i>et al.</i> 2006)	<i>In vitro</i>	Coronal	Fluid filtration	Immediate	140
(Verissimo <i>et al.</i> 2007)	<i>In vitro</i>	Apical	Dye	7 days	70
(Duggan <i>et al.</i> 2009)	Animal (dog)	Coronal	Bacterial	16 weeks	66
(Moura-Netto <i>et al.</i> 2009)	<i>In vitro</i>	Apical	Dye	Immediate	60
(Nawal <i>et al.</i> 2011)	<i>In vitro</i>	Coronal	Bacterial	30 days	40

Table 1.2: Studies reporting no significant differences between Resilon™ and gutta-percha in preventing apical and coronal leakage

Reference	Type of study	Apical or coronal seal tested	Leakage model	Duration	Sample size
(Tay <i>et al.</i> 2005a)	<i>In vitro</i>	Apical	Dye	3 hours	20
(Pitout <i>et al.</i> 2006)	<i>In vitro</i>	Coronal	Bacterial & dye	72hrs & 3months	56
(Silveira <i>et al.</i> 2007)	<i>In vitro</i>	Apical	Dye	Immediate	108
(Fransen <i>et al.</i> 2008)	<i>In vitro</i>	Coronal	Bacterial	56 days	73
(Kocak <i>et al.</i> 2008)	<i>In vitro</i>	Apical	Dye	3 days	55
(Hollanda <i>et al.</i> 2009)	<i>In vitro</i>	Coronal	Bacterial	60 days	40
(Kazandag <i>et al.</i> 2010)	<i>In vitro</i>	Coronal	Glucose penetration	60 days	100

Table 1.3: Studies showing the superiority of gutta-percha to Resilon™ in preventing apical and coronal leakage

Reference	Type of study	Apical or coronal seal tested	Leakage model	Duration	Sample size
(Onay <i>et al.</i> 2006)	<i>In vitro</i>	Apical	Fluid filtration	Immediate	70
(Shemesh <i>et al.</i> 2006)	<i>In vitro</i>	Coronal	Fluid filtration & glucose penetration	8 weeks & 56 days	120
(Pasqualini <i>et al.</i> 2007)	<i>In vitro</i>	Apical	Bacterial	47 days	88
(De-Deus <i>et al.</i> 2008a)	<i>In vitro</i>	Coronal	Fluid filtration	14 months	40
(Hirai <i>et al.</i> 2010)	<i>In vitro</i>	Apical	Fluid filtration	Immediate	64
(Santos <i>et al.</i> 2010)	<i>In vitro</i>	Coronal	Fluid filtration	Immediate & 180days	82

A proportional increase in the volume/surface area ratio leads to an increase in the stress value and in certain instances may be high enough to debond the adhesive interfaces (Scherrer *et al.* 2010). It is acknowledged that a flowable composite can produce stress relief in resin restorations (Eick *et al.* 1993, Carvalho *et al.* 1996); this however is dependent on both geometry of the cavity and resin film thickness. A great deal of effort has been made to reduce the polymerization shrinkage of dental composites and has led to the introduction of low-shrinking composites (Cramer *et al.* 2011). For example, in a class I box-like cavity, there are five cavity walls for bonding and only one surface is unbonded. The ratio of bonded to unbonded areas is called the C-factor (Van Ende *et al.*

2012). In the root canal space, there is only a very small unbonded surface area. Hence, only minimal stress relief by resin flow can take place.

Further concern about Resilon™ is that polycaprolactone, one of its main components, is known to be biodegradable. It has been demonstrated that Resilon™ is susceptible to degradation when exposed to an alkaline environment and enzymes such as lipase and cholesterol esterase (Tay *et al.* 2005b, Tay *et al.* 2005c). Incomplete resin infiltration of the root dentinal tubules by mild self-etching adhesive is a further challenge associated with bonding of Resilon™ to root canal dentine as smear layer formation may cover some parts of the root canal walls (Tay & Pashley 2007). Previous studies have demonstrated other unfavourable limitations of Resilon™, including low bond strength between Resilon™ and its sealer (Ungor *et al.* 2006, Sly *et al.* 2007). The possible reason for the low bond strength in Resilon™ based-obturations could be the low concentrations of dimethacrylates or absence of free radicals within Resilon™, producing a fragile and questionable coupling with its resin sealer (Epiphany) (Burtscher 1993, Gesi *et al.* 2005). It is suggested that the remaining radicals play a significant role on the adhesion of any composite materials as the post cure temperature of the composite may lead to reduce the half-life of radicals which in turn compromise bonding to the composites (Burtscher 1993). Moreover, the tensile strength and the stiffness of Resilon™ might be too low to reinforce endodontically treated roots (Williams *et al.* 2006). A later study confirmed that the adhesive procedures of currently available endodontic obturation materials are not able to strengthen the roots (Grande *et al.* 2007).

1.3.4.3 Polyurethane-based root canal obturation material

This material is a polymer based root canal obturation material which was developed in 2008 in combination with a visible light curable root canal sealer

(Hsieh *et al.* 2008, Lee *et al.* 2008). The chemical constituents of the central core material are predominantly zinc oxide (70% ZnO) as a filler and thermoplastic polyurethane (30% TPU) as a matrix, whereas the root canal sealer consists of urethanacrylate/tripropylene glycol diacrylate (UA/TPGDA). An earlier study demonstrated that TPU exhibits good abrasion resistance, tear strength, shock absorption, and biocompatibility (Boretos & Pierce 1967), and showed the effectiveness of the segmented polyurethane when used in a variety of biomedical devices. The polyurethane-based obturation system was introduced to improve the bond strength between the core material and the root canal sealer via modification of the urethane group (-NHCOO-) with Polybutyleneadipate (PBA) as both of UA/TPGDA sealer and the main chain of TPU possess -NHCOO- and PBA. Another reason for the improved bond strength could be the formation of hydrogen bonds between the hydrogen and nitrogen atoms on the urethane groups of UA/TPGA sealer and ZnO/TPU cone. These hydrogen bonds may underpin the adhesion of sealer to the cone material (Hsieh *et al.* 2008). The polyurethane-based root canal obturation materials showed a slight increase in elastic modulus and tensile strength in comparison to gutta-percha and Resilon™ (Hsieh *et al.* 2008), However, the apical sealing of the canal space is also potentially compromised due to the risk of incomplete light curing of the resin in the most apical region.

1.3.4.4 *Phosphate glass polycaprolactone-based composite*

A polycaprolactone (PCL)–iron phosphate glass (P-Glass) composite was developed in 2009 as a root canal obturation material, which is able to release ions forming a precipitate on its surface, enhancing the sealing ability of the material (Alani *et al.* 2009). The polycaprolactone-based composite contains polycaprolactone as a matrix and iron phosphate glass ($\text{CaO–Na}_2\text{O–Fe}_2\text{O}_3\text{–P}_2\text{O}$) as filler. The main rationale for use of such material for root canal obturation is that its bioactivity might improve the sealing of the root canal space (Hench & Paschall 1973, Kasuga 2005). It is believed that a great potential for hard and soft tissue engineering is offered by phosphate glasses (P-glasses), which are a unique group of bioactive materials. The composite of P-glass and PCL is capable of releasing calcium and phosphate ions along with iron and sodium in an aqueous medium; this may promote antimicrobial effects (Zehnder *et al.* 2004). The material is used without any sealer; this might lead to difficulties in the obturation of irregularities, such as isthmuses and fins of the root canal space because of the limited flowability.

1.3.4.5 *Bio-gutta root canal obturation material*

Bio-gutta is a composite material used for root canal obturation (Bio-Gutta; Smartodont llc, Zurich, Switzerland), which has been developed recently by incorporation of a nanometric 45S5 bioactive glass into the matrix polymer of gutta-percha, polyisoprene (Mohn *et al.* 2010). Such composite materials showed good immediate sealing properties when heated. Incorporation of bioactive glass fillers into the composite material makes it bioactive and significantly improves its sealing ability. Mareending *et al.* (2013) showed that bio-gutta possesses several promising basic properties such as the release of alkaline species in aqueous environment and self-adhesiveness to radicular dentine (Mareending *et al.* 2013).

However, in order for this material to be commercialised, it needs further investigations in terms of biocompatibility, possible antimicrobial activity and nature of the self-adhesiveness.

1.3.5 Root canal sealers

Root canal sealers are used to fill the interface between the core material and the root canal wall, the voids inside the core material and the accessory canals, to serve as a lubricant and to obtain an hermetic apical seal (Skinner & Himel 1987). The distribution of the sealer in root canals is variable and is determined by the technique used to obturate the root canal (Wu *et al.* 2000). Ørstavik (2005) carried out a literature review which demonstrated that sealers play an important role in sealing the root canal system with entombment of remaining microorganisms and obturation of inaccessible areas of prepared canals (Ørstavik 2005).

Microbial growth and persistent infection may be found in dentinal tubules and lateral canals (Love & Jenkinson 2002). A root canal sealer must therefore be able to penetrate the dentinal tubules, which cannot be filled by the core obturation material (Saleh *et al.* 2004). Ricucci and Siqueira (2010) reported that strategies other than finding a technique that better squeezes sealer or gutta-percha within lateral canals and apical ramifications should be pursued to effectively disinfect these regions (Ricucci & Siqueira 2010). The antimicrobial action of the sealer is another important property to help kill residual bacteria and inactivate their toxins either by direct or indirect contact with the microbes (Heling & Chandler 1996). The ideal properties of a root canal sealer were outlined by Grossman (Grossman 1988) as shown in box 1.

Box-1

Properties of an ideal sealer

- Exhibits tackiness when mixed to provide good adhesion between it and the canal wall whilst setting.
- Establish a hermetic seal
- Sufficiently radiopaque
- Very fine powder so it can mix easily with the liquid
- No shrinkage on setting
- No staining of tooth structure
- Bacteriostatic
- Exhibits a slow set
- Insoluble in tissue fluid
- Biocompatible
- Retrievable

Root canal sealers in use today can be classified into two groups based on their constituents, namely eugenol-containing sealers and non-eugenol containing sealers as described in box 2.

Box-2

Types of sealer

- Eugenol-containing sealers (Zinc oxide-eugenol sealers)
- Non-eugenol-containing sealers
 - Calcium hydroxide-based
 - Resin-based
 - Glass ionomer-based
 - Silicon-based
 - Calcium silicate-based [Mineral Trioxide Aggregate (MTA)]

1.3.5.1 Zinc Oxide-Eugenol sealers

ZnO eugenol sealers are widely used and considered as a standard against which other sealers are assessed. The active ingredient is mostly liquid eugenol and the powder contains zinc oxide that is finely sifted to enhance the flow of the cement (Cohen & Hargreaves 2006). A natural resin is usually added to the powder in attempt to yield the paste a smoother texture. In some types of ZnO eugenol sealers, silver or barium or even bismuth salts are added to ensure their radiopacity. The radiopacity of the ZnO eugenol sealers correspond to 4-5 mm of aluminium as the standard in accordance with ANSI/ADA (2000) (ANSI/ADA 2000), which is lower than that of gutta-percha (6.22 mm) (Beyer-Olsen & Ørstavik 1981).

ZnO eugenol sealers are relatively weak and porous upon setting, and are susceptible to decomposition in tissue fluids when extruded into the periapical tissues. Ørstavik *et al.* (2001) showed that ZnO eugenol based sealer shrank 0.3%-1% over 4 weeks storage in water. It was concluded that bacterial penetration may be associated of a sealer shrinkage as little as 1% (Ørstavik *et al.* 2001). The requirements for compliance with the standard were therefore fixed at a linear expansion of not more than 0.1% or shrinkage of not more than 1%. Previous studies reported that ZnO eugenol sealer may improve the sealing ability of gutta-percha points when eugenol comes into contact, leading to a small volumetric expansion of gutta-percha due to a sort of chemical interaction occurring between eugenol and the components of gutta-percha point (Michaud *et al.* 2008). However, this expansion is not standardised and depends on the powder/liquid ratio of the sealer itself. In addition, internal voids within gutta-percha may be generated because of the gutta-percha expansion. Cytotoxicity

caused by free eugenol that is present in freshly mixed sealers is a further drawback of ZnO eugenol sealers.

Generally, the components of ZnO eugenol sealer are based on Grossman's formula as shown in box-3.

Box-3

<u>Zinc Oxide-Eugenol based sealer (Grossman sealer)</u>	
<u>Powder</u>	<u>Liquid</u>
Zinc oxide	Eugenol
Staybelite resin	
Bismuth subcarbonate	
Barium sulphate	
Sodium borate	

1.3.5.2 *Calcium hydroxide-based sealers*

Calcium hydroxide-based sealers have been developed with the aim of preserving the vitality of the pulp tissue stump and stimulating healing with hard tissue formation at the root apex. Calcium hydroxide is soluble and may weaken the remaining tooth structure with long-term exposure (Cvek 1992). An *in vitro* study showed no significant difference in the sealing ability of calcium hydroxide and zinc oxide-eugenol sealers (Jacobsen *et al.* 1987).

1.3.5.3 *Resin-based sealers*

The first resin sealer developed was AH 26 (Dentsply DeTrey, Konstanz, Germany). It consists of an epoxy resin base which sets slowly when mixed with an activator. The benefits of AH 26 include adhesive properties, good sealing, and antibacterial activity (Limkangwalmongkol *et al.* 1992). Nonetheless, severe

inflammatory reactions and paraesthesia were reported after overextension with AH 26 and gutta-percha (Barkhordar & Nguyen 1985, Ørstavik & Mjor 1988) due to the release of formaldehyde (Spangberg *et al.* 1993). Due to its undesirable effects, AH 26 was modified and replaced by AH Plus (Dentsply DeTrey), which is a two paste system formulated to polymerise with no release of formaldehyde with lower cytotoxicity and lower solubility.

1.3.5.4 *Glass ionomer-based sealers*

Glass ionomer sealers were introduced in the endodontic field immediately after their development as a restorative material (Pitt Ford 1979). The main benefit of glass ionomer sealers over the traditional sealers is their ability to adhere to root dentine.

1.3.5.5 *Silicon-based sealers*

A slight expansion upon setting, good sealing ability, excellent biocompatibility, and high radiopacity are the main advantages of the resin-based sealers (Wu *et al.* 2002).

1.3.5.6 *Bioceramic-based root canal sealer*

For endodontic use, bioceramic-based materials have been developed, mainly as repair cements (Leal *et al.* 2011) and as root canal sealers (Loushine *et al.* 2011). The chemical composition is a combination of calcium silicate and calcium phosphate. Bioceramics are biocompatible, effective antimicrobial agents, non-shrinking upon setting and are capable of forming hydroxyapatite during the setting process and create a bond between dentinal wall and obturation material (Zhang *et al.* 2009a, Loushine *et al.* 2011). Bioceramic sealers (BCS) are hydrophilic in nature and the moisture in the dentinal tubules promotes their setting reaction. Therefore, the setting time is dependent on the presence of

moisture in dentinal tubules and may range from 4 hours to 10 hours (Hess *et al.* 2011). The bioceramics are pre-mixed and injectable composed of zirconium oxide, calcium silicate, calcium phosphate, calcium hydroxide, filler, and thickening agents. Several brands of BCS are available such as EndoSequence BC Sealer (Brasseler, Savannah, GA, USA) and iRoot SP (Innovative Bioceramix, Vancouver, Canada). Research has indicated that the apical sealing of BCS with gutta-percha in a single cone technique was superior to that of gutta-percha condensed using the continuous wave condensation technique (Zhang *et al.* 2009b).

Mineral Trioxide Aggregate based root canal sealer-filler

Mineral Trioxide Aggregate (MTA) was introduced in dentistry in 1993 by Torabinejad (Parirokh & Torabinejad 2010), and approved for endodontic use in 1998. The benefits of MTA include biocompatibility, osteogenicity, and hard tissue formation (Chen *et al.* 2009, Hakki *et al.* 2009, Reyes-Carmona *et al.* 2009). Parirokh & Torabinejad (2010) concluded that MTA is a bioactive material that influences its surrounding environment.

During the setting process, the pH of the MTA substantially increases from an initial level of 10.2 to 12.5. This high alkalinity is associated with its antibacterial activity (Duarte *et al.* 2003). Other properties of MTA include stimulating cementum-like tissue formation (Holland *et al.* 2002) alongside osteoblastic adherence (Favieri *et al.* 2008) and bone regeneration (do Nascimento *et al.* 2008). Thus, MTA can be effectively used as a therapeutic agent in a variety of applications, namely direct pulp capping (Min *et al.* 2008), pulpotomy (Barrieshi-Nusair & Qudeimat 2006), repair of internal (Meire & De Moor 2008) and external root resorption (Gonzales & Rodekirchen 2007), repair of perforations (Yildirim & Dalci 2006), apexification (Chueh *et al.* 2009) and apexogenesis (root-end

induction in immature apices) (Jung *et al.* 2008) in immature pulpal necrotic teeth, root-end obturation (Saunders 2008), and as a sealer/filler obturation material (Bogen & Kuttler 2009). Chemically, MTA is a fine hydrophilic powder which consists primarily of dicalcium silicate, tricalcium silicate, tricalcium aluminate, gypsum, tetracalcium aluminoferrite, and bismuth oxide. It is a hydraulic silicate cement that can set and be stable under water, which distinguishes it from the old dental silicate cements which depended on reaction with phosphoric acid (e.g. acid-base system) (Fridland & Rosado 2005, Darvell & Wu 2011).

MTA is derived from Portland cement; however the introduction of bismuth oxide increases its radiopacity. Heavy metal constituents (Monteiro Bramante *et al.* 2008), lack of adequate radiopacity (Bortoluzzi *et al.* 2009), significant setting expansion (Islam *et al.* 2006), high solubility (in some forms), and broad distribution in particle size are unfavourable properties of Portland cement. Currently, MTA is available in two forms; grey and white which are slightly different in their chemical composition as grey MTA includes mineral traces which are responsible for the dark appearance of the cement. In addition, levels of tetracalcium aluminoferrite in white MTA are less than those of grey MTA. The initial setting of MTA demands approximately three to four hours. It has been reported that after three months of MTA placement, calcium hydroxide would have been slowly released to reduce blood flow by activating a pyrophosphatase enzyme, and then inducing hard tissue formation (Roberts *et al.* 2008). Although MTA possesses lots of benefits and applications in endodontics, it still has certain undesirable points. Tooth discoloration is an unfavourable effect caused by a grey MTA, particularly when placed in proximity of the cemento-enamel junction in anterior teeth (Asgary *et al.* 2005). Retreatability of MTA root canal obturation is another potential problem, particularly in curved canals (Boutsioukis *et al.* 2008).

It is also believed that the alkalinity of the calcium silicate material might affect the mechanical properties of dentine (Sahebi *et al.* 2012, Sawyer *et al.* 2012). Another potential disadvantage of MTA is its slow setting time (2.5-4) hours for an initial set and 14-21days for complete setting (Torabinejad *et al.* 1995). A new calcium silicate-based material was introduced in an attempt to circumvent the disadvantages of MTA, namely Biodentine (Septodont, Saint-Maur-des-Fossés, France). It contains tricalcium silicate, calcium silicate, calcium carbonate and oxide, iron oxide, and zirconium oxide as its powder components whilst liquid components include calcium chloride and water-soluble polymer (Han & Okiji 2011).

1.3.6 Obturation techniques and current philosophies for successful endodontics

Obturation is the final stage of the endodontic treatment aimed at achieving coronal and apical seals, sealing the lateral and accessory canals, and consistently obturation all the pathways along the root canal system. Root canal obturation was described by American Association of Endodontists as “the three-dimensional obturation of the entire root canal system as close to the cementodentinal junction as possible” (American Association of Endodontists 1994). It is reported that biofilms and their toxins initiate, maintain, and exacerbate periapical disease (Takehashi *et al.* 1965, Nair 2004). In order to prevent or treat the disease process, root canal treatment should eliminate or reduce the microbes to as a low level as possible. Thus the main purpose of the obturation phase of endodontic treatment is to provide a fluid tight seal along the root canal space to prevent leakage of residual toxins and bacteria into the periapical tissues. Plentiful techniques for root canal obturation are currently available ranging from cold lateral condensation to vertical warm condensation, thermoplasticized gutta-percha systems, carriers coated with alpha phase gutta-percha, flowable obturation materials. However, clinical trials failed to identify significant differences between outcomes of teeth obturated with different techniques (Hoskinson *et al.* 2002, Peters *et al.* 2004). Part of the present research is concentrated on the carrier-based gutta-percha obturation methods.

Carrier-based obturation of root canals was first described by Ben Johnson in 1978 (Johnson 1978), and Thermafil® (Dentsply Tulsa Dental, Tulsa, OK) was introduced as a carrier-based obturation material with a solid core during the 1990s. Obturators were designed to correspond to the ISO standardized file sizes and to tapered nickel-titanium rotary files. Originally, the carrier was made of

stainless steel and later replaced with plastic material. The plastic carriers of current Thermafil® obturators are made up of different materials depending on their size. Smaller sizes (up to size #40) use Vectra (Ticona GmbH, Sulzbach, Germany), which is polyethylene terephthalate, a liquid crystal polymer insoluble in solvents, and larger sizes use polysulfone, which is soluble in chloroform (Parker & Glickman 1993). It has been reported that both polysulfone and polyethylene terephthalate aromatic polyesters have carcinogenic effects due to the presence of benzene ring in their chemical structure. Carrier-based obturation systems include Thermafil®, Dens-Fil (Dentsply Maillefer, Tulsa, OK), Soft-Core (Axis Dental, Coppel, TX), and resin-based obturators (RealSeal One; SybronEndo, Orange, CA). The common problems reported by clinicians using core-based obturation systems include the lack of apical control (Gutmann *et al.* 1993), the inability to fill oval-shaped canals (De-Deus *et al.* 2008b), and the difficulty associated with removal of the carrier in case of retreatment (Ibarrola *et al.* 1993, Zuolo *et al.* 1994, Wesselink 2003, Gutmann *et al.* 2006). Contemporary carriers include grooves to facilitate the removal of the carriers; however, retrievability remains a concern, and the most recently introduced obturators such as the cross-linked gutta-percha (GP) obturators (GuttaCore, Dentsply Tulsa Dental) have been designed to facilitate removal by simply trephining through the core.

A composite material (30% polypropylene with 70% barium sulphate (Flex Point NEO; Neo Dental Chemical Products, Tokyo, Japan) has also been proposed as a potential carrier-material (Kato & Nakagawa 2010) with a temperature tolerance of about 148°C. However, the material has a high component of barium sulphate as a radiopacifier, which does not assist in the stiffening of the polymer matrix and acts only as a filler to impart radiopacity. This research attempts to develop

new carrier-based core materials for carrier-based root canal obturation that could be retreated effortlessly.

Thermafil® (Tulsa, OK) system was introduced as a carrier-based obturation material with a solid core during the 1990s. The carrier acts as a plunger via applying a gentle pressure to push the root canal obturation material into the irregularities of the root canal, lateral and accessory canals. Industrially, obturators have been originally designed to correspond ISO standardized file sizes alongside the tapered nickel titanium rotary files. Several studies have found that the Thermafil® obturation system provides a good hermetic and tight apical seal superior to that of lateral condensation (Gulabivala *et al.* 1998, Gencoglu *et al.* 2002, Gencoglu 2003) while other studies have found the lateral condensation technique to be superior (De Moor & Hommez 2002, Gatewood *et al.* 2004). Extrusion of material beyond the apical constriction of the preparation is a common disadvantage of thermoplasticised carrier-based systems (Kytridou *et al.* 1999, Robinson *et al.* 2004). Retrievability of the plastic carrier is another concern, particularly with smaller sizes (Ibarrola *et al.* 1993).

SuccessFil (Akron, OH, USA) is a three dimensional injectable thermoplastic gutta-percha system based on utilizing a gutta-percha syringe system, with the pre-measured carrier length placed into the syringe prior to extrusion of the gutta-percha onto the carrier and subsequent insertion into the canal. Various pluggers are used to condense gutta-percha around the carrier, depending on the canal morphology. Essentially, the system includes the SuccessFil gutta-percha syringe which contains 7mL of high viscosity gutta-percha used in combination with a SuccessFil core to fill the canal in 3D. The solid core obturation is made of titanium alloy. This technique offers flexibility with respect to the shape and amount of gutta-percha extruded onto the carrier. A previous study demonstrated

that the apical seal in mandibular first molar in dogs obturated with SuccessFil technique was superior to that of Thermafil® obturation system (Ulbricht *et al.* 2002). Another study demonstrated that Ultrafil exhibited a much better apical seal than that of obtura and lateral condensation (Olson *et al.* 1989).

SimpliFill (Discus Dental, Culver City, CA, USA) is the only carrier-based obturation system that features a removable carrier. It is often performed following canal preparation using Light-speed instruments. Simplifill exhibits a cold carrier-based technique based on removing the carrier from the root canal space once the coating obturation material has been delivered by the carrier. Only the apical portion of the carrier is coated with gutta-percha or Resilon™. The unfilled coronal and middle thirds of root canal space can then be filled with further gutta-percha or Resilon™. This sectional technique is efficient, and leakage potential is similar to other obturation methods. An *in-vitro* study of Simplifill technique showed a superior apical seal compared with other techniques and a reduced treatment time required (Santos *et al.* 1999, Stein *et al.* 2004). Other investigators showed that the microbial coronal seal of canal obturated with Simplifill is significantly superior to that of the conventional obturation techniques tested (lateral condensation, warm vertical condensation and obtura II) (Shipper & Trope 2004).

1.3.6.1 Coronal seal & apical seal

It is generally accepted that the endodontic treatment outcome is significantly correlated with the technical quality of the root canal obturation (Nair 2006). Tronstad *et al.* (2000) found that the technical quality of root canal obturation was more important than the technical quality of coronal restoration (Tronstad *et al.* 2000). Some studies suggested that a positive outcome of root canal treatment may be established even in presence of inadequately obturated root canals,

when the quality of the coronal restoration is sufficient (Ray & Trope 1995, Trope *et al.* 1995a, Kirkevang *et al.* 2000). A systematic review and meta-analysis indicated that the odds for healing of apical periodontitis increase with both adequate root obturation and adequate restorative treatment (Gillen *et al.* 2011).

1.3.6.2 Reinforcing effect of root canal obturation material

As previously noted, endodontically treated teeth are more liable to fracture than teeth with vital pulps (Reeh *et al.* 1989). Root fracture is one of the most common causes of extractions of endodontically treated teeth (White *et al.* 2002, Kishen 2006). A root canal obturation material with a modulus of elasticity closer to that of radicular dentine might help increase the fracture resistance of endodontically treated roots. Dentine is a collagen-rich organic matrix reinforced by calcium phosphate mineral particles. It exhibits an elastic modulus of about 16,000 MPa (Kinney *et al.* 1999). Thus, in principle, composite materials used to fill the root canal should have a modulus of elasticity closer to that of dentine to prevent root fracture. The stiffness of the material itself may help in resisting the masticatory forces (Williams *et al.* 2006, Shanahan & Duncan 2011). Adhesive materials such as GICs (Cobankara *et al.* 2002, Lertchirakarn *et al.* 2002), epoxy resin-based sealers (Cobankara *et al.* 2002), dentine bonding agent (Zidan & ElDeeb 1985), polymethylmethacrylate-based resins (Imai & Komabayashi 2003) and urethane dimethacrylate (UDMA)-based sealer (Teixeira *et al.* 2004) have been proposed to improve both the root canal sealing ability and root reinforcement. However, some studies showed that the bond strength between the adhesive root obturation materials and root canal wall is not sufficient to strengthen endodontically treated teeth.

1.3.6.3 Tri-dimensional obturation

One of the keys to establish long-term success of endodontic treatment is to thoroughly obturate the disinfected root canal space in three dimensions (Epley *et al.* 2006, da Silva Neto *et al.* 2007). Several investigators reported that a complete entombment of the residual micro-organisms by using an appropriate root obturation material could help establishing a long-term success of endodontic treatment (Leduc & Fishelberg 2003, Ørstavik & Pitt Ford 2008).

Micro-computed tomography (μ -CT) is used to observe root canals in three dimensions. It is used for the evaluation of root canal anatomy, assessment of root canal morphology after instrumentation, volumetric measurements of root canal obturation (Jung *et al.* 2005, Barletta *et al.* 2008), and for *in vitro* assessment of root canal obturation (Hammad *et al.* 2009).

1.3.7 Experimental polymer composites developed as obturation core materials in this research study

1.3.7.1 Aliphatic polyester-based composites

Polyesters include polymers with ester functional groups in the polymer backbone. They may be defined as long-chain polymers chemically composed of at least 85% by weight of an ester, a dihydric alcohol and a terephthalic acid. Esters are formed by the reaction of an alcohol group with a carboxylic acid group as shown in Figure 1.5. The chemical units linking ester groups can be varied over a wide range, making the polyesters a diverse group providing multiple benefits (Edlund & Albertsson 2003). Recently, biodegradable polymers derived from renewable sources were introduced in biomedical sciences (Zhang & Thomas 2011).

The term “Biodegradation” refers to the ability of the polymer to be primarily depolymerised via hydrolysis or enzymatic processes (Göpferich 1996). In order

to achieve this, the polymer sites which are capable of being hydrolysed must be accessible to an alkaline environment. Theoretically, all the polyesters are potentially degradable in the presence of water that causes cleavage of the main chain ester bonds because of the reversible reaction of the esterification process. Practically, the hydrophobicity of the aromatic polyester prevents water approaching the labile ester bond; in the meantime, only aliphatic esters will degrade over time (Edlund & Albertsson 2003).

Polyesters are divided into aromatic, semi-aromatic, and aliphatic. The aromatic and semi-aromatic polyesters are considered to be degradation-resistant polymers due to their rigid rod-like chains such as polyethylene terephthalate and polyamides. Thus aliphatic polyesters were employed in the current research study as biodegradable polymers for endodontic purposes. Poly-L Lactic Acid (PLLA) and Polyhydroxybutyrate (PHB) are the synthetic biodegradable polymers used in this study. These polymers are also thermoplastic and capable of being processed and formed into films and fibres. Synthetic biodegradable aliphatic polyesters are primarily fabricated by industrial polymerisation of monomers such as glycolic acid (PGA), lactic acid (PLA), butyric acid (PHB), valeric acid (PHV) and caprolactone (PCL). Such polymers and their copolymers have been widely used in different applications in medicine and dentistry. The use of synthetic absorbable polymers in biomedicine is not limited to wound closures alone. Other uses include fracture fixation plates (Higashi *et al.* 1986) , ligating clips and grafts used in cardiovascular surgery (Daniel & Olding 1984), bowel anastomosis (Hardy *et al.* 1985), nerve growth conduits (Mackinnon *et al.* 1985) and in embolization therapy (Kumar & Good 1986).

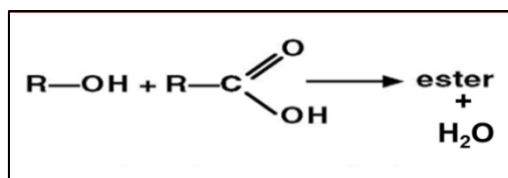


Figure 1.5: Chemical formula of esters family

Poly(lactic acid (PLA)

Poly-L-lactic acid (PLA), $-\text{[CH(CH}_3\text{)COO]}_n$, is a highly translucent, tough material. It can be processed with a large number of techniques and is commercially available in a wide range of grades. It exhibits interesting properties in terms of biodegradability and biocompatibility, which makes it to be suitable for different biomedical applications (Av  rous 2008). It is superior to other biodegradable aliphatic polyesters in terms of thermal and mechanical properties and transparency of the processed material (Urayama *et al.* 2003). PLLA is an aliphatic thermoplastic polyester, which is being extensively researched to replace conventional petrochemical-based polymers. Lactic acid is converted to the dimer, lactide which is purified and polymerised to polylactic acid. Poly (L-lactic acid) (PLLA) is the product resulting from the polymerisation of L-isomers and poly(D-lactic acid) (PDLA) is formed by polymerisation of D-isomers. The polymers rich in the L-form are more crystalline and those which are rich in the D-form are more amorphous.

Recently, several PLA-based technologies were developed with an emphasis on achieving chemical, mechanical, and biological properties equivalent or superior to conventional polymers (Rasal *et al.* 2010). Originally, polylactic acid was obtained from the polymerization of lactic acid derived from the fermentation of natural sugars from corn, beets, or sugar cane. The physicomechanical properties of PLA were reported by several investigators, showing a crystallinity of 37%, melting temperature (T_m) at around 173-178  C, glass transition

temperature (T_g) between 60-65°C, and a tensile modulus between 2.7 and 16 GPa (Fiore *et al.* 2010). Polylactic acid can be processed into fibres and films using extrusion, injection moulding, and spinning technologies. It is reported that polylactic acid polymers can be used as matrices for ceramic particles or as adjuvants to calcium phosphate cements and as carriers for drug delivery (Bose & Tarafder 2012). A tailored biodegradation/drug release to the ceramic material was produced using these polymers (Habraken *et al.* 2007).

Polyhydroxybutyrate (PHB)

The persistent problems associated with managing the plastic waste materials have led to development of biodegradable plastics. (PHB), $[\text{CH}(\text{CH}_3)\text{CH}_2\text{COO}]_n$, is a biodegradable semi-crystalline thermoplastic polymer, developed to replace expensive and non-biodegradable polymers. PHB is a biocompatible material, suitable to be used as a tissue-engineering scaffold (Rathbone *et al.* 2010) and it is synthetically produced by bacterial fermentation via the condensation of two molecules of Acetyl-CoA to give acetoacetyl-CoA which is subsequently reduced to hydroxybutyryl-CoA, which is in turn used as a monomer to polymerize PHB (Williams *et al.* 1999). PHB is one of the most attractive aliphatic polyesters, since it can undergo biodegradation in various environmental conditions without forming toxic products (Griffin 1993, Tokiwa & Ugwu 2007). However, PHB is brittle and its lack of flexibility has limited its application (Lootz *et al.* 2001). To secure greater flexibility, the degree of crystallinity must be reduced. It has been suggested that blending of PHB with other polymers can improve its physical and mechanical properties, leading to higher flexibility and higher elongation at breaking point (Qiu *et al.* 2003, Chen & Wu 2005). The thermal properties of PHB were characterised by differential scanning calorimetry: the glass transition temperature being around 2°C and the melting point around 173°C (Lee *et al.*

2002b). The tensile strength of PHB was reported to be approximately at around 25-40 MPa.

1.3.7.2 Polyolefins

In the beginning of the 20th century, polyolefins; polyethylene (PE) and polypropylene (PP) were invented and became increasingly used in modern life. Polyethylene $[-CH_2-CH_2-]_n$ is a thermoplastic polymer with long hydrocarbon chains formed by polymerisation of ethylene gas, $CH_2=CH_2$. Using a high temperature and a high pressure in the polymerisation process produces a polyethylene with low density whereas high density polyethylene is formed by performing a lower temperature and a lower pressure with active catalyst processes (Brydson 1999). Petrochemicals and dehydration of ethanol are the main sources of ethylene production (Whiteley *et al.* 2000). The extent of branching with molecular weight and crystal structure of polyethylene determines significantly its mechanical properties, in which hydrogen atoms are linked to a carbon backbone in linear or branching forms. The branched versions are known as low density polyethylene (LDPE) as shown in Figure 1.6A, whereas the linear versions are known as high density polyethylene (HDPE) as shown in Figure 1.6B. Polyethylene, is available in different grades ranging from high density polyethylene (HDPE), medium density polyethylene (MDPE), to low density polyethylene (LDPE). Based on its density and branching, polyethylene is classified into different categories:

- Ultra-high-molecular-weight polyethylene (UHMWPE)
- Ultra-low-molecular-weight polyethylene (ULMWPE)
- High-molecular-weight polyethylene (HMWDPE)
- High density polyethylene (HDPE)
- High density cross-linked polyethylene (HDXLPE)

- Cross-linked polyethylene (XLPE)
- Medium density polyethylene (MDPE)
- Linear low density polyethylene (LLDPE)
- Low density polyethylene (LDPE)
- Very low density polyethylene (VLDPE)

It is suggested that aromatic hydrocarbons, for instance xylene or toluene, are capable of dissolving polyethylene at high temperature (75-90°C).

Due to their hydrophobic chain and high molecular weight, polyolefins are considered inert materials. The success of polyolefins use in different fields is mainly attributed to their relative ease of fabrication, low cost, desirable flow properties, potential flexibility, low surface free energy, strong hydrophobicity, excellent resistance to chemicals, good biocompatibility and good biostability (Wang & Bonfield 2001). Thus, Polyethylenes were used in this research study due to their desirable biological, physical and mechanical properties as shown in box 4. LDPE category was employed in this research for endodontic applications as it is quite flexible, processible, and biocompatible.

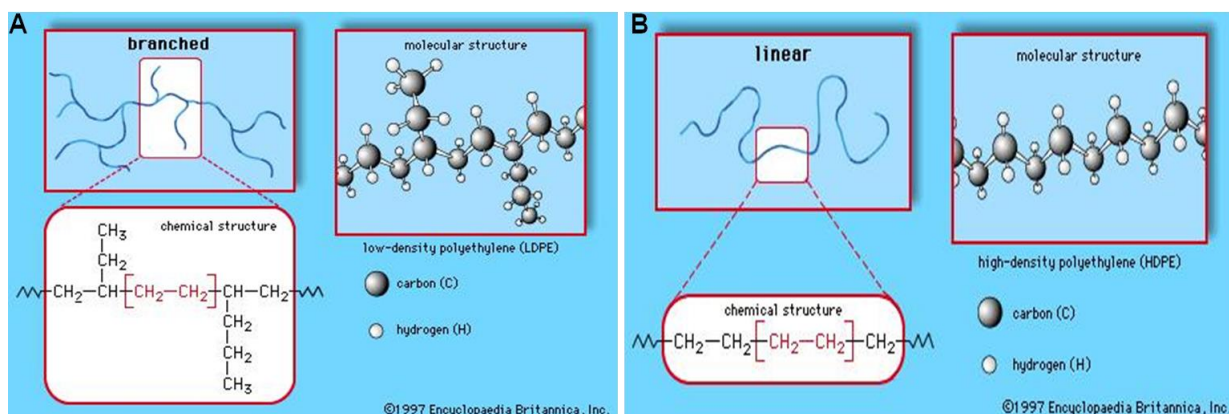


Figure 1.6: A) branched form of polyethylene, low density polyethylene, B) linear form of polyethylene, high density polyethylene (adapted from <http://www.britannica.com/EBchecked/media/125939/The-linear-form-of-polyethylene-known-as-high-density-polyethylene> Retrieved 27 January 2014)

Box-4

Property	Low Density Polyethylene (LDPE)	High Density Polyethylene (HDPE)
Melting point	~ 115°C	~ 135°C
Crystallinity	Low crystallinity (50-60%), main chain contains many side chains of 2-4 carbon atoms	High crystallinity (90%) contains less than one side chain per 200 carbon atoms in the main chain
Flexibility	More flexible than HDPE due to lower crystallinity	More rigid than LDPE due to high crystallinity
Strength	Not as strong as HDPE due to irregular packing of polymer chains	Strong as a result of regular packing of polymer chains
Transparency	Good transparency since it is more amorphous than HDPE	Less transparent than LDPE because it is more crystalline
Density	0.91-0.94g/cm ³ lower crystalline than HDPE	0.95-0.97g/cm ³ higher density than LDPE
Chemical properties	Chemically inert insolvent at room temperature in most solvent, good resistance to acids and alkalis	Chemically inert

Several investigators showed that incorporation of hydroxyapatite filler particles into polyethylene resulted in the production of a composite which could be used as bone analogue material (Bonfield 1988, Deb *et al.* 1996, Nazhat *et al.* 2000), and for middle ear implants (Tanner 2010). In the endodontic field, a certain flexibility of the obturation material is required due to the complexity of the root canal space. The quantity of the ceramic fillers would help obtaining the required flexibility. In the present work, hydroxyapatite (HA) reinforced low density polyethylene composites (HA/LDPE) were successfully synthesised as potential materials for endodontic use. Thus, hydroxyapatite & 45S5 Bioglass[®] were used in the study to reinforce low density polyethylene with strontium oxide (SrO) to achieve the radiopacity required by an endodontic material.

1.3.7.3 Fillers used in the present study

Hydroxyapatite (HA): HA has the simple chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, with an ideal molar ratio of calcium to phosphorus (Ca/P) of 1.67 and a hexagonal crystal structure. Because it is one of the major components of the human body, HA is safe, comprising 60% of bone, 97% of tooth enamel and 70% of tooth dentine. HA is also present in saliva and can help remineralising incipient carious lesion (Cury & Tenuta 2009). Owing to its safety and biocompatibility, HA is one of the most widely used bioceramic materials in the biomaterial field and tissue engineering (Supova 2009). It is used in a wide range of medical and dental applications such as artificial tooth and bone, tissue repair, and coatings to improve the biocompatibility of orthopaedic implants (Park *et al.* 2010). It is worthwhile to mention that HA has attracted attention as an implantable calcified tissue replacement material. A randomized in situ study showed that medical nano HA can inhibit the development of caries and remineralise early carious lesions (Najibfard *et al.* 2011). They concluded that nano-hydroxyapatite

dentifrice produced remineralisation comparable to that of fluoride dentifrice. HA is one of the few materials that are being classified as bioactive, being able to enhance bone ingrowth and osseointegration when used in orthopaedic, dental and maxillofacial applications (Oosterbos *et al.* 2002). Thus, HA may enhance the healing process of diseased tissues by stimulating the growth of bone tissue (Ben-Nissan & Choi 2006). It is also believed that HA provides suitable sites for osteoblast cell attachment (Zhang *et al.* 2007). They showed that hydroxyapatite reinforced polyethylene supported osteoblast attachment, proliferation and differentiation. Hydroxyapatite undergoes ion substitution as it is not uncommon for non-stoichiometric hydroxyapatites to exist. Thus the hydroxyl group of HA tends to be replaced by other ions such as carbonate, fluoride and chloride. Nonetheless, HA is brittle as all other ceramic materials, it is not capable of resisting long load bearing applications and this limits its use in clinical settings (Cordell *et al.* 2009). The strategy to address the brittleness of HA is to introduce a polymeric component (Bonfield 1988, Ural *et al.* 2000). Several studies focused on changing the morphology of porous HA bioceramic through incorporating whiskers in an attempt to toughen the HA matrices and enhance their biological response (Bose *et al.* 2009, Fang *et al.* 2013). A silane coupling agent is commonly used to improve the interfacial bond strength between polymer and filler (Deb *et al.* 1996).

Bioactive glass filler (45S5 Bioglass®): Bioglass® is a family of bioactive glasses based on silicate systems containing silica (SiO₂), disodium oxide (Na₂O), calcium oxide (CaO) and phosphate (P₂O₅) (Hench & Andersson 1993). 45S5 Bioglass® was discovered later by Hench and his co-workers. Bioglass® is bioactive, biocompatible, osteoconductive, nontoxic, non-inflammatory, and can be effectively used in biomedical applications, such as maxillofacial

reconstruction, dental applications, coatings for orthopaedic prostheses and as filler for bone defects (Misra *et al.* 2006, Rezwan *et al.* 2006). Nano-composites of Bioglass[®] have been clinically used as scaffolds for periodontal tissue regeneration (Peter *et al.* 2010) and for dentine-pulp regeneration (Bae *et al.* 2012). Furthermore, 45S5 Bioglass[®] exerts a considerable antibacterial effect in particular, the antibacterial effect of Bioglass[®] is attributed to the high aqueous pH value and to the formation Bioglass[®] debris on the bacterial surface (Allan *et al.* 2001, Hu *et al.* 2009). Bioglass[®] is able to bond to both hard and soft tissues by formation a deposition of thin layer of hydroxycarbonate apatite (HCA) on the glass surface when it comes in contact with biological fluids *in vitro* and *in vivo* (Hench 1998). This bone-bonding behaviour is an example of “bioactivity”. Several studies proved that the result of the rapid generation of a thin layer of HCA produces a bond formation on the glass surface that it is similar to that of biological apatite (Pereira *et al.* 1994, Hench *et al.* 1998, Jones *et al.* 2006). It has been found that the HCA phase that forms on the surface is chemically and structurally equivalent to the mineral phase in bone, providing interfacial bonding (Hench 1998). Brittleness is considered to be an undesirable property of bioactive glass and other ceramic materials (i.e. HA), making them unsuitable in load-bearing applications.

1.4 Materials engineering categorisation

1.4.1 Metals

Metallic products have been widely used in biomedical applications such as hip prostheses or dental implants. Metallic plates, pins, and screws have also been used for bone fixation (Holt 1986, Steflik *et al.* 1993). Implants are usually made up of stainless-steel, cobalt-chrome alloys and titanium alloy. Metallic implants are bio-inert and unable to exhibit osseointegration function, therefore coating

with bioactive ceramic particles (i.e. hydroxyapatite) was carried out to improve implant integration, allowing bone ingrowth (de Groot *et al.* 1987, Bryington *et al.* 2013, Salman *et al.* 2013). Negative tissue response is another drawback issue of metallic implants due to undesirable ion release from the metallic devices, interfering with the nature of living tissues. Retrievability is also a serious complication of the temporary metallic implants which may require using complicated surgical intervention (Lorbach *et al.* 2008).

1.4.2 Ceramics

Ceramics are inorganic, non-metallic solid materials fabricated by heat application and subsequent cooling. They may be crystalline or partly crystalline or even amorphous. Advanced technology in ceramics has led to the development of a promising group of materials, the “bioceramics”. The development of bioceramics started with metallic oxides such as Al_2O_3 and MgO (Black 1981), calcium phosphate (i.e. hydroxyapatite, tricalcium phosphate) (LeGeros 1991) and glass ceramic (i.e. bioglass) (Hench *et al.* 1971). Calcium phosphate and glass-ceramic are considered to be bioactive ceramics that have the ability to elicit a positive response on the biological environment, whilst the metallic oxides are entirely bioinert. Synthetic hydroxyapatite is one of the bioactive ceramics currently and commonly used in biomedical disciplines as a bone analogue material however, it is too brittle to resist high-load when implanted in areas subjected to high load. Therefore, hydroxyapatite particles are incorporated into the resin matrix as particulate fillers generating a reinforced polymer composite (Bonfield *et al.* 1986, Knowles *et al.* 1992).

1.4.3 Polymers

Polymers are widely used in a vast range of applications that far exceeds that of any other class of material available to man. Current applications range from

packaging, coatings, adhesives to textile and industrial fibres, composites, and biomedical devices. Two main routes of improving polymer properties were suggested by either incorporating particulate fillers or fibres into their matrices creating polymer composites. They are extensively used in conjunction with fillers in the dental field. Degradability and physico-mechanical behaviours are considered to be detrimental issues for polymer composites. In the endodontic field, it is reasonable to recommend using thermoplastic, flexible and non-degradable polymers such as polyethylene and polypropylene as potential root canal filling components. However, biodegradable polymers may be used in some cases of endodontic obturation; particularly in root canals obturated with carrier-based systems. In other words, the carrier material can be constructed with degradable polymer (i.e. PLA) which may facilitate retreatment.

1.4.4 Polymer matrix composites

A composite material is a multi-phase material composed of two or more chemically distinct phases, which are normally a polymer resin as a matrix (continuous phase) in conjunction with the dispersion phase which may be particulate or fibrous (Work *et al.* 2004). The use of polymer matrix composites for biomedical and dental applications offers clinically relevant benefits, allowing for instance an easy removal of the implant when required if degradable polymers are used. The lack of leaching of metallic ions from the implants is another benefit associated with the use of polymer matrix composites. Particulate fillers and fibres were used to reinforce polymer materials generating versatile composites and potentiating the material's bioactivity (Masuelli 2013).

1.4.4.1 Fibre reinforced composites

Ceramic and non-ceramic fibres (bioactive glass fibre, carbon fibre, polyethylene fibre) are frequently used to reinforce polymers, creating specifically-designed

composites with suitable mechanical and physical properties for the required application (Evans & Gregson 1998, De Santis *et al.* 2000). The stiffness and the strength of the composites can be tailored and optimised by employing various resin matrices in combination with fibres such as carbon fibre reinforced polyetheretherketone (Hancox 1982). Furthermore, the behaviour of the composite generated can be modulated to be either completely degradable or partially degradable or may be even non-degradable, depending on the type of polymers and fibres used (Dagang *et al.* 2007). Besides that, the bioactivity of the composite can be improved by incorporating bioactive fibres, for instance glass fibres, into the polymer matrices (Hench *et al.* 2004, Mohammadi *et al.* 2011). Nevertheless, fatigue fracture is much more expected to take place at the polymer/fibre interface than in the polymers with no fibre reinforcements (Latour & Black 1993). It would be highly recommended to use physical and chemical modifiers via creating a good wettable surface (contact angle $< 90^\circ$) at the interface in an attempt to improve the compromised interfacial bonding strength of fibre reinforced polymers (Ekstrand *et al.* 1987). Generally speaking, fibre reinforced composites have better mechanical properties than that of non-reinforced composites. Fundamentally, fibre reinforced composites may include three different types of fibres; short fibres, long fibres and continuous fibres. Considering the mechanical properties and processing technology of fibres, short fibres tend to be manufactured and mixed easily but the strength and the stiffness is inferior to that of long or even continuous fibres.

Consequently, an application of short fibre reinforced composites is being limited in high load bearing areas. Apparently, several issues contribute in detecting the effectiveness of fibre reinforced composites such as length, form, and direction of

fibres. The adhesion of fibres to the polymer matrix is also a challenge to face in producing a durable fibre reinforced composite (Chung *et al.* 1998, Vallittu 1998).

1.4.4.2 Filler reinforced composites

Composites reinforced with particulates have been increasingly used in different disciplines including medicine and dentistry. The main benefit of incorporating particulate fillers is the reinforcement of polymer matrices and subsequent improvement in their mechanical properties (i.e. elastic modulus) (Bonfield *et al.* 1986). Bioactivity improvement and decrease in creep of the composite is a further benefit associated with the incorporation of fillers (Bonfield *et al.* 1986, Castaldini & Cavallini 1986). Radio-contrast can also be manipulated by adding a quantity of filler particle to the matrix.

The degradation rate of biodegradable polymers can be potentially modulated through the management the amount of filler incorporated. van der Meer *et al.* (1996) pointed out that the degradation behaviour of polylactic acid was significantly changed by the addition of ceramic filler (van der Meer *et al.* 1996).

1.4.5 Possible methods to strengthen the interface phase in the composites

1.4.5.1 Self-reinforced composites

Self-reinforcement of composites is a chemical treatment method of enhancing the adhesion between polymer and filler using fibres of the same chemical structure as the polymer matrix. Therefore, the bond strength between the polymer and the filler of the composite is substantially improved. Polylactic acid (PLA), polyglycolic acid (PGA) and polymethyl methacrylate (PMMA) fibres have been increasingly used to fabricate self-reinforced composite (Tormala *et al.* 1991, Ashammakhi & Rokkanen 1997, Wright *et al.* 1998)

1.4.5.2 Plasma treatment of fibres

Treatment of the fibre surfaces by gas plasma technology has led to substantial improvement in the interfacial bond strength between resin matrix and fibres. The principle of this method is to remove surface contaminants and subsequently create polar groups on the surface of the fibres with a more wettable surface at the interface of fibre and matrix and thus generating a chemical covalent bond on the surface (Kolluri *et al.* 1988). Argon, O₂, methane and CO₂ are most commonly used for the treatment of fibres.

1.4.5.3 Silane coupling agents

Silanation is the treatment of the surface of filler particles through self-assembly with silane coupling agents to make it more compatible and dispersible in the polymer, serving as a reinforcing additive. A chemical bond (-Si-O-Si-) is formed across the polymer-filler interface by displacing alkoxy groups on the silane with hydroxyl groups of mineral components (Mohsen & Craig 1995) as shown in the Figure 1.7.

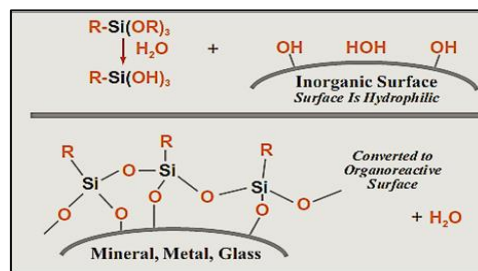


Figure 1.7: Treatment of a filler surface by an organosilane, (adapted from www.Xiameter.com 2009)

This results in improving the durability of the composite material via creating a more physicochemical stable material may be expected (Arksornnukit *et al.* 2004). Thus, the main benefits of saline treatment are reported in the following box 5.

- Modifying the adhesion between the filler and matrix
- Improving dispersion of the filler in the polymer resin
- Increasing wet-out of the filler by the polymer resin
- Increasing mechanical characteristics
- Reducing the viscosity of the filler/polymer mix

As the polyethylene used in this present work is non-polar and hydrophobic and the hydroxyl group on the surface of HA filler is hydrophilic, creation of a polar group is therefore essential for achieving affinity between the polyethylene and the filler by using a coupling agent (Deb *et al.* 1996). Coupling agents play an important role in enhancing the polymer/filler interaction as they promote molecular bonding, providing modification in the interfacial strength (Xavier *et al.* 1990). 3-(methacryloxy) propyltrimethoxysilane (A-174) is the silane coupling agent used in this study as an organo-functional silane to treat the surface of HA filler.

1.4.6 Polymer processing innovation technology

Polymers can be defined as long chain molecules consisting of many repeating units, and the monomers are the smallest repeating units in the polymer chain. The oligomer is a polymer with relatively few structural units. The polymer chains are often made up of covalently linked carbon atoms (Combe *et al.* 1999). The reaction by which polymers are prepared from monomers is called polymerisation and the degree of polymerisation is the number of repeating monomers in the polymer. Depending on their behaviour when heated, polymers are generally categorised into thermoplastics, which are able to be softened and shaped at temperatures above the glass transition temperature and can be moulded

whereas, the second polymer group is thermosetting polymers, which can decompose upon heating without showing a glass transition.

The glass transition temperature is the temperature at which the polymer converts from the glassy state to rubbery state making its molecules easy to flow. The molecular weight (MW) is a further important property of polymers, which is equal to the degree of polymerisation multiplied by the molecular weight of the repeating unit. Polymers with high molecular weights have a greater rigidity and strength, and higher values of glass transition temperature and melting temperature than polymers with low molecular weights. A number of polymer processing techniques were suggested such as extrusion including single and twin screw, injection moulding, compression moulding, transfer moulding and blow moulding (Vlachopoulos & Wagner 2001). The selection of a processing technique is mainly dependent on the nature of polymers being processed, and whether thermoplastic or thermosetting.

Extrusion is the polymer processing technique used in the current study, which is considered to be the most common processing technology. Extrusion is the process of converting a solid feedstock into a homogeneous melt and pressurising it through a die at a uniform rate along the extruder. Following the die, careful drawing forces are required to obtain the desired shape with the correct molecular orientation of the materials extruded (McCrum *et al.* 1997), as shown in Figure 1.8. Extrusion is a two-part process including, plastication and shaping. Plastication stands for the material extrusion along the extruder through a die whereas shaping confers the final shape to the material.

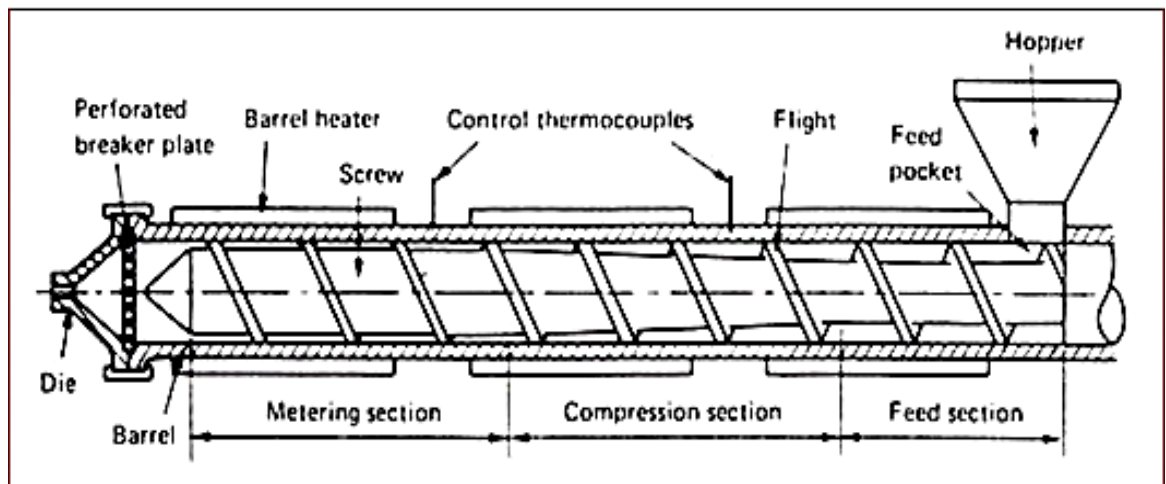


Figure 1.8: Schematic representation showing the extrusion process (adapted from McCrum *et al.* 1997)

Chapter 2 Materials and Methods

This chapter provides an insight about the materials employed in the current study and their processing methods and describes the characterisation methods used. A schematic of the production process of polymer composites is shown in Figure 2.1.

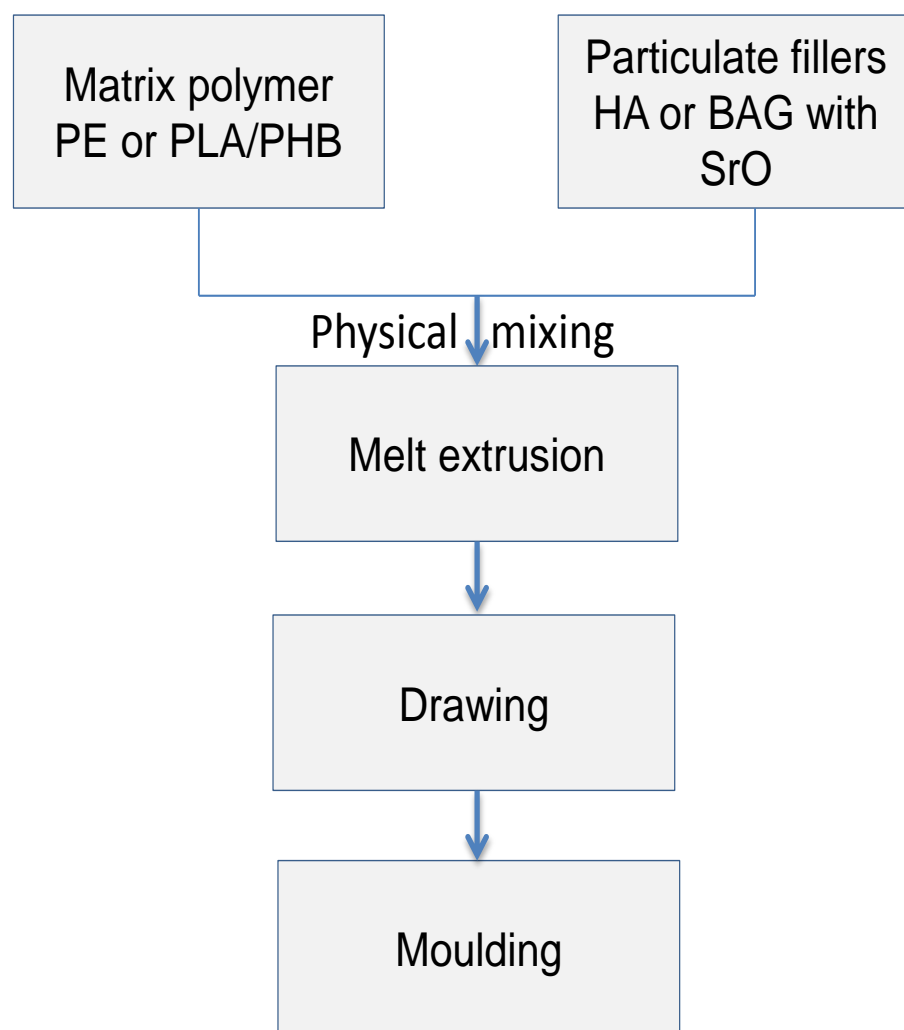


Figure 2.1: Flowchart of the polymer composites production process

Polymers of poly (3-hydroxybutyrate) PHB and poly (l-lactic acid) PLLA were selected for use as matrices for composite production. PHB, (50µm & Mw 500.000) (Biomer, Germany) and PLLA, (177µm & Mw 80.000) (Purasorb, pL-18,

Purac Biochem BV Gorinchem, Netherland) were purchased as powders. Polyethylene with a low density of 0.917 g/cm^3 (Good Fellow Chemical Products, Huntingdon, UK) (LDPE) was used as the polymer matrix in combination with fillers to generate composites for endodontic application. Two fillers, namely hydroxyapatite and Bioglass[®] with a mean particle size of 3-5 μm and 45-80 μm , respectively were used in this study. The filler particles of either HA or Bioglass[®] particles were blended with SrO to impart the radiopacity required for any root canal obturation material. The concentration of fillers was optimised taking into account the handling considerations of the material for endodontic use. For core carrier endodontic use as “carrier”, two filler concentrations of 20% and 40% by weight of HA were used to fabricate the composites LDPEHASRO20 and LDPEHASRO40. For obturation core material, HA and Bioglass[®] fillers with concentrations of 20% and 30% by weight were used to fabricate the composites.

2.1 Silanation of hydroxyapatite filler particles

HA particles were treated with the A-174 silane coupling agent, 3-Trimethoxysilyl propyl methacrylate, (Merck, Frankfurt, Germany) using a procedure described by Deb *et al* in 1996. In brief, the silane coupling agent, A-174, was dissolved in a 70/30 acetone/water mixture. HA particles were added to the resultant solution and stirred with a magnetic stirrer at room temperature for 1 hour. The temperature was gradually raised while stirring to 100°C and maintained for 1 hour to obtain a slurry. Thereafter, the HA slurry was placed in an oven and maintained at 125°C for 2 hours. The coated HA was washed with water, centrifuged, and dried at 37°C temperature. The silanated HA particles aimed to improve the bond strength between the polyethylene and the filler at their interface achieving a more stable and durable composite.

2.2 Processing of composite fibres using melt-extrusion method

The composites were prepared via melt extrusion using a 12-mm single screw extruder (Rondol Technology, Staffordshire, UK). The processing temperature of the extruder was monitored depending on the type of polymer used. The speed of the extruder was monitored depending on the type of polymer used. The speed of the rotary screw was optimized between 20-25 rpm with continuous feeding to obtain a consistent output of the composite and minimize the risk of thermal degradation. The materials were extruded through a die drawn down to different diameters and tapers as shown in Figure 2.2.

In the case of the polyethylene composite, the polyethylene was produced in the form of granules, which led to inadequate mixing with the filler phase, thus re-extrusion was carried out. A suitable mill was not available for enabling the grinding of the LDPE, especially as it is a soft material and causes static in conventional mills. Thus, in order to obtain a homogenous composite, the fibres fabricated in this study were re-extruded. The fibres obtained from the extruder were stored in a desiccator for further analysis.

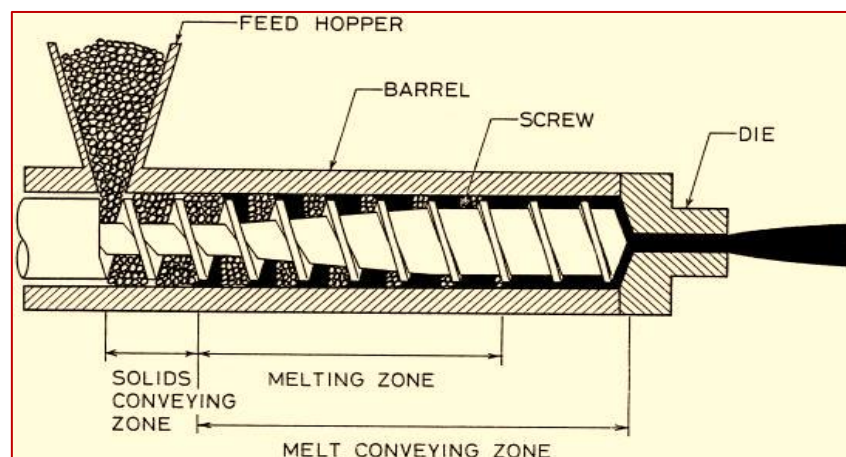


Figure 2.2: Single screw extruder

2.2.1 Processing of polylactide/polyhydroxybutrate-hydroxyapatite composite fibres for endodontic use (core-carrier)

Before extrusion to form the composite fibres, the raw materials were weighed and mixed manually. A variety of composite fibres were fabricated in the current study using a hot melt extrusion process. Pure PHB and PLLA fibres were first fabricated and considered as control groups. Blends of PHB and PLLA in different ratios without fillers, and PHB/PLLA with hydroxyapatite filler were also prepared as shown in Table 2.1. The melting temperatures of polyesters, PLLA and PHB were pre-determined using the data from differential scanning calorimeter analysis. Accordingly, the processing temperature of polyester-based composite fibres was maintained between 170-180°C. Screw rotation speed was optimized at 25 rpm with continuous feeding. The materials were then extruded through a 1.5mm diameter die and further drawn down to a diameter of approximately 0.5-1.3mm with the help of a moving coil. The experimental fibres were then stored in a desiccator until characterisation.

Table 2.1: The polyester matrices of PLLA, PHB and their blends

Product	Composition
PLLA	PolyL-Lactic Acid
PHB	PolyHydroxyButyrate
PHB50	Polyhydroxybutyrate 50% + polyL-Lactic acid 50%
PHB50/HA10	Polyhydroxybutyrate 50% + polyL-Lactic acid 50% + hydroxyapatite10%
PHB50/HA20	Polyhydroxybutyrate 50% + polyL-Lactic acid 50% + hydroxyapatite20%
PHB20	Polyhydroxybutyrate 20% + polyL-Lactic acid 80%
PHB20/HA10	Polyhydroxybutyrate 20% + polyL-Lactic acid 80%+ hydroxyapatite 10%
PHB20/HA20	Polyhydroxybutyrate 20% + polyL-Lactic acid 80%+ hydroxyapatite 20%

Experimental obturator fabrication:

The experimental composite fibres shaped as carriers were successfully coated with gutta-percha to form the carrier-based root canal obturator. The alpha phase GP (Obtura Spartan, CA, USA) was dissolved in chloroform until a viscous liquid was obtained. A cylindrical rubber tube with a stopper (16-mm length with different thicknesses) was prepared to receive the composite fibre. GP was then injected using an extremely fine needle into the tube, creating an evenly distributed layer of GP around the carrier.

2.2.2 Processing of polyethylene-based composites for endodontic applications

2.2.2.1 Core carrier for carrier-based root canal obturation

The necessary amounts (Table 2.2) of LDPE and silanated HA with SrO were weighed, mixed then placed in the hopper of the 12-mm single screw extruder. The processing temperature was maintained at 150-160°C. The speed of the rotary screw was optimized based on a pilot study and 25 rpm was used for the processing. Two different feed compositions were compounded to fabricate the fibres as shown in the Table 2.2. The materials were then forced through a die drawn down to different diameters and tapers.

Table 2.2: Polyethylene containing hydroxyapatite composites fabricated as carriers

Product	Composition
LDPESHASRO 20	Low density polyethylene 70%+ silanated hydroxyapatite 20%+ strontium oxide 10%
LDPESHASRO 40	Low density polyethylene 50% + silanated hydroxyapatite 40% + strontium oxide 10%

2.2.2.2 Obturation core root material

i) Polyethylene hydroxyapatite composites

The composite fibres for the obturation core material were fabricated in a similar manner as described in Section 2.2.2.1. The concentrations of the composite constituents were varied in order to optimise the properties suited to an obturation core material. Four groups of composite fibres were fabricated as shown in Table 2.3.

Table 2.3: Polyethylene hydroxyapatite composites fabricated as obturation core material

Product	Composition
LDPESHASRO 20/30	Low density polyethylene 50% + silanated hydroxyapatite 20% + strontium oxide 30%
LDPENSHASRO 20/30	Low density polyethylene 50% + non-silanated hydroxyapatite 20% + strontium oxide 30%
LDPESHASRO 30/20	Low density polyethylene 50% + silanated hydroxyapatite 30% + strontium oxide 20%
LDPENSHASRO 30/20	Low density polyethylene 50% + non-silanated hydroxyapatite 30% + strontium oxide 20%

ii) Polyethylene Bioglass[®] composites

45S5 Bioglass[™] with a particle size distribution of 45-80 µm was added to the polyethylene matrix to generate a second set of composites. Two composite fibres were fabricated as shown in Table 2.4 and optimised using single screw extruder at a processing temperature between 150-160°C and rotary speed around 20-25 rpm.

Table 2.4: Polyethylene containing Bioglass[®] composites fabricated as obturation core material

Product	Composition
LDPEBAGSrO 20/30	Low density polyethylene 50% + bioactive glass 20% + strontium oxide 30%
LDPEBAGSrO 30/20	Low density polyethylene 50% + bioactive glass 30% + strontium oxide 20%

2.3 Characterisation methods of the experimental composites

2.3.1 Fourier transform infrared spectroscopy (FTIR)

Infrared spectroscopy (ATR accessory, Spectrum One, PerkinElmer, Waltham, MA, USA) was employed to characterise the chemical composition of the test materials, confirming the incorporation of hydroxyapatite or Bioglass[®] fillers into the polyethylene matrix. The specimens of the composite fibres were heated at different temperatures depending on the relevant melting point of the materials used and then pressed into small discs suited for recording the infrared spectra. The silanation of hydroxyapatite particles was also confirmed by FTIR spectroscopy.

2.3.2 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC, Perkin Elmer, Waltham, MA, USA) was employed to measure thermal properties of the experimental materials in terms of glass transition, melting, and crystallization temperatures. The test material was placed in a special aluminium pan, whilst the reference was an empty aluminium pan with no material in it. Both the sample and the reference were maintained at the same temperature over the experiment. For each of the composites two heating and two cooling cycles were recorded to determine the thermal properties. By observing the difference in heat flow between the sample and reference, DSC scans are able to measure the amount of heat absorbed (endothermic) or released (exothermic) during the phase transitions. The glass transition temperature was measured from the midpoint of S-shaped transition slopes using the DSC software.

2.3.3 Dynamic Mechanical Analysis (DMA)

A dynamic mechanical analyser (DMA 8000, Perkin Elmer) was used to measure the changes of mechanical behaviour under dynamic conditions as a function of temperature, time, frequency, stress, pressure or a combination of these parameters. DMA is a powerful tool to characterise the elastic modulus and damping (tan delta) properties of the test materials under cyclic load at different frequencies and at different temperatures. In DMA measurements, a portion of the energy stored elastically is called “storage modulus” whilst a portion of the energy lost in the form of heat is named loss modulus of elasticity. So, the modulus of elasticity obtained from DMA is equal the storage modulus plus the loss modulus, and the ratio of loss modulus to storage modulus at a defined temperature is the tan delta, which refers to the damping property of the materials. DMA technique can be used with six common modes of action including: single cantilever, dual cantilever, tension, compression, shear and 3-point bending. The single cantilever bending mode was used in this work to characterise the samples. All samples were tested at a frequency of 1 Hz, and heated at different temperatures at a rate of $3^{\circ}\text{C min}^{-1}$. The samples with predefined dimensions were directed in the alignment of the DMA device and the data collected from the software of the DMA.

2.3.4 Tensile test

The elastic modulus and tensile strength of the experimental materials at room temperature were determined by a Universal Testing Machine (Model 5569 A; Instron, High Wycombe, UK). The crosshead speed was set at 5 mmmin^{-1} as specified by the American Society for Testing and Materials (ASTM) Standard D-412-98a for rubber materials (ASTM International 2002). Dog bone-shaped specimens were prepared, for the experimental composite materials;

polylactide/polyhydroxybutyrate-based composite, and polyethylene-based composite and the control materials (gutta-percha for core material and Thermafil[®] carrier for core carrier system), by melting and pressing the test and control materials in a standardized aluminium mould (gauge length = 7.5 mm, width = 2.25, and depth = 1.85 mm) at different temperatures depending on the melting point of the materials used for the test; 150°C for polyethylene-based composite, 180°C for the polylactide/polyhydroxybutyrate-based composite, 70°C for gutta-percha and 350°C for the polysulfone as gutta-percha carrier of Thermafil[®] system.

2.3.5 X-ray contrast

The radiopacity of the experimental materials was assessed using dental radiography equipment (Planmeca Oy, Helsinki, Finland) in combination with a phosphor plate digital system and a grey-scale value aluminium step wedge, which represents the different shades between black and white varying from 0 to 255 pixels, where 0 represents black and 255, represents white. The grey-scale measurement was then converted into mm equivalents of aluminium. The aluminium type used was 1100 alloy. The exposure parameters were set up at 70kV, 8mA and 0.2 s. The object to-focus distance was 30 cm. The degree of radiopacity was determined using an aluminium washer, 1 mm thick with an internal diameter of 10 mm, filled and compressed with the test material and radiographed together with the graduated aluminium step wedge, with thicknesses varying from 1 mm to 10 mm in steps of 1 mm each (ANSI/ADA 2000). The radiopacity of the test materials inside the washer was compared with the steps of the aluminium step wedge on a computer screen using image J software (Image J processing and analysis in java, version 1.47g).

2.3.6 Scanning electron microscopy (SEM)

SEM observations using a Hitachi S-3500N microscope (Hitachi High Technologies, Tokyo, Japan) were also performed at different magnifications (500x and 900x) to scrutinise the homogeneity of the experimental composites developed in this study. Samples of 2mm long and 1.5mm thick were prepared for SEM examination and the dispersion of fillers within the polymer matrix was observed. The field of micrograph vision was 0.50 μm .

2.3.7 Biocompatibility evaluation

The cytocompatibility of the experimental composites was assessed using primary osteoblast like cells (HOB obtained at passage number² 18) taken from human alveolar bone. The cell culture was obtained at 37°C in a humidified atmosphere with 5% CO₂ in Dulbecco's modified eagles medium (DMEM). Specimens of the experimental composites were prepared by melting the composites to produce discs of diameter of 12mm to fit a 24 well-plate.

2.3.7.1 MTT (Methyl tetrazolium) assay

The MTT (Methyl tetrazolium), is a colorimetric assay for measuring the viability of cells and their metabolic function, based on the fact that the yellow colour of MTT salt (Sigma, UK) is reduced and converted to a purple formazan product by mitochondrial dehydrogenase which can be translated as a measure of cell number. This enzyme is only present in intact, living cells. In this assay, the test materials were placed into labelled containers (bijou) using sterile forceps. Subsequently, 3 mL of sterile DMEM (Sigma, UK), supplemented with 10% foetal calf serum (FCS) were added. The containers were sealed with a parafilm and placed onto a roller mixer (Luckham 4RT, Burgess Hill, UK) for 24 and 72 hours

² "Passage number" refers to the number of times cells have been transferred from vessel-to-vessel

(elution time). The eluants (supernatant) were moved to a new labelled bijou in a sterile environment and then stored at -20°C for cell culture measurements. In this assay, osteoblast-like cells were exposed to the test eluants for 24 h and 72 h. Trypsinization of the osteoblast-like cells in 10 mL of DMEM media was carried out and the number of the cells was counted and seeded with HOB cells at a density of 1×10^4 cells/100 μ L per well. 100 μ L of the suspended cells was added to a 96-well plate and left in an incubator for approximately 1 day at 37°C in humidified air with 5% CO₂ in order to allow cell confluence. The 100 μ L of media from the well plates were removed by pipetting and 100 μ L of the eluants were added to designated wells. The plates were then placed into the incubator and assessed at 24h and 72h cell exposure time. Standard culture medium was used as a negative nontoxic control and 10% ethanol diluted in media as a positive control. After the required exposure time, the fluid from the 96-well plates was removed by tipping the plates over a wad of tissue. 100 μ L of pre-prepared MTT solution was added to each well and placed in the incubator for 4 hours. Then 100 μ L of Dimethyl sulfoxide (DMSO), cryoprotectant to prevent cell death, was added to each plate. The plates were then covered with foil and placed on a shaker for approximately 5 minutes. The viability of cells was then determined by measuring the absorbance of the solution at 570 nm test wavelength and 630 nm reference wavelength using a microplate reader (Dynex technologies, San Diego, US).

2.3.7.2 alamarBlue™ cell proliferation assay

The principle of this test is based on the use of alamarBlue™ as a dye indicator. In this assay, both colour changes and fluorescence are expected to occur in response to the chemical reduction of culture medium, which results from cell growth and division. Meanwhile, the alamarBlue™ can be removed and replaced

with fresh medium so that monitoring can be kept on. Thus, the polymer-based composites were also assessed for biocompatibility using alamarBlue™ assay. This assay was used to measure the proliferation of cells. Trypsinizing and counting osteoblast cells were conducted in 10 mL of cell suspension with the same methodology used for the MTT assay. Seeding of cells with $1 \times 10^5 \text{ mL}^{-1}$ was carried out. The test materials were placed into the 24-wells plate using sterile forcep. Then 1 mL of cell suspension was added to the wells and incubated for a six time points: 1, 3, 7, 14, 21 and 28 days in standard culture conditions of 5% CO₂ in humidified air at 37°C.

At day 1, the media was removed from the wells and discarded. Then 100 µL media were mixed with 1 mL of alamarBlue™, diluted 1:10 in phenol red-free, solution (Serotec) and incubated for 4h at 37°C; 5% CO₂. Wells without any cells were used as the blank control whilst Thermanox™ was used as non-toxic negative control (cells and media). Following the incubation, 100 µL from each well were taken and transferred to a new 96-well plate. Absorbance was measured on a fluorescent plate reader at 570 nm test wavelength and 630 nm reference wavelengths. Afterward, the 96-well plates were washed three times to remove the blue dye and 1 mL media was replaced for further measurements at 3, 7, 14, 21, 28 days.

2.3.8 Interaction of the experimental composites with simulated body fluid and alkaline media

Using an *in vitro* model, the degradation of the experimental composites fibres was conducted in two different immersion media namely Simulated Body Fluid (SBF) and Hypocal® medicament (Ellman International, Oceanside New York, USA) which is non-setting calcium hydroxide paste as alkaline media. The average weight of the samples was pre-determined and 2mL of immersion

medium was used and placed in a glass vial. The fluid uptake was measured and recorded at regular intervals. The wet fibres were quickly blotted with a filter paper to remove excess moisture and weighed accurately within 1 minute on a Mettler Toledo® AT 250 digital analytical/semi-micro balance (Mettler-Toledo, Laboratory & Weighing Technologies, Greifensee, Switzerland) with a readability of 0.0001g (four digits). Two samples were used for each study group.

2.3.9 Physical integrity of the experimental composites after sterilisation with gamma irradiation

The experimental polymer composite fibres developed in this study were assessed for sterilisation by gamma irradiation as a robust sterilisation method. The dimensions and weight of the composite fibres (n=5) were standardised at fixed values and then subjected to gamma sterilisation. The fibres were evaluated for any changes in their weight and dimensions after sterilisation using an accurate balance and digital calliper tool (Maplin Electronics, Rotherham, UK) respectively.

2.3.10 Thermocouple temperature measurements

The temperature rise within the root canal space was recorded using a thermocouple system via obturation of the root canal space with system B heat obturation system used to soften and condense the gutta-percha at the apical region of root canal (SybronEndo Corporation, Orange, CA, USA). The intra-canal temperatures reached with gutta-percha and the experimental hydroxyapatite-polyethylene composite were measured and compared. An extracted single rooted tooth with a length of 16mm was enlarged apically to a size #35 K-flex file at the established working length using a standard step-back technique and then shaped to receive both gutta-percha (control) and the experimental LDPE/HA root canal obturation. The prepared tooth was embedded

in a transparent acrylic block. Then the embedded tooth was sectioned in a coronal-apical direction into two halves using an Isomet saw. In the acrylic block, four holes with suitable screws and nuts were created and placed to facilitate removal of the obturation material and clean it to receive further obturation materials for multiple use. Five intra-canal holes of 0.5mm width were prepared from the external surface of the block in each tooth half, 1mm from the apical part of the root extend to the coronal third, holes distance was set at 3mm (1, 4, 7, 10, 13) mm. A thermocouple device provided with a 5 channel wire was connected to the tooth via the intra-canal holes.

2.3.11 Fluid filtration technique

Thirty simulated canals and thirty single-rooted human extracted teeth with round canals were selected for this study with consent of the appropriate ethical institutional review board (Research Ethics Committee, Reference Number 10/H0804/056). Roots with cracks and perforations were not included in the study. The teeth were carefully cleaned with curettes to remove the soft tissue remnants and disinfectant in sodium hypochlorite and then stored in saline solution before instrumentation. The specimens used in this study (extracted teeth and simulated canals) were instrumented to the working length with a size #30 K-file using the standard step-back technique, while the coronal third of each root was prepared with a 2-3 gates glidden bur (Dentsply, Maillefer, Switzerland). A total of 10 mL of 2.5% sodium hypochlorite (NaOCl) was used to flush the root canals of the extracted teeth and the simulated canal. Subsequently, the canals were obturated with the GuttaCore carrier-based root canal obturator system (Dentsply Tulsa, Johnson City, USA) and with the experimental LDPE/HA carrier-based obturator system with or without the use of the Tubliseal Sealing agent (Orange, CA, USA). The specimens were glued to a Perspex™ (Perspex

Distributions Ltd, London, UK) platform (2 cm × 2 cm × 0.5 cm) that was perforated by an 18 gauge stainless steel tube. Each specimen was connected to a hydraulic fluid filtration system able to deliver a hydrostatic water pressure *via* a nitrogen gas pressure (10 psi/69 kPa) and polyethylene tubing through the coronal end of the roots. A 25 mL capacity micro-capillary tube (Microcaps, Fisher Scientific, Atlanta, GA, USA) was positioned above a millimetre ruler and horizontally between the pressure reservoir and the crown segment. The sealing ability of the tested materials applied in human and simulated root canals was evaluated at different intervals time (30, 60 and 90 minutes).

2.3.12 Micro-Computed Tomography (μCT)

High-resolution μCT is an emerging technology with several promising applications in many different fields of dentistry including endodontics. μCT has been used for both volumetric measurements and 3D reconstructions of root canals before and after obturation. μCT with high resolution (6.5 μm) was used in the present study for two purposes. The first objective was to quantify the thickness and distribution of gutta-percha around the carrier material for the carrier-based root canal obturation system. The second objective was to measure the percentage volume of voids within the root canal space filled with the experimental LDPE/HA obturator and to compare it with the commercial ones. The obturators were scanned using a GE Locus SP μCT scanner (General Electric, London Ontario, Canada); with an X-ray tube voltage of 80kVp and a current of 80 μA. A 0.1 mm aluminium filter was used to attenuate the X-ray source. The specimens were immobilised using cotton gauze and scanned to produce 6.5x 6.5x 6.5μm voxel size volumes. All scans were imported using Scan IP (Simple ware®, Exeter, UK).

2.3.12.1 *μCT evaluation of the experimental HA/PE and commercial obturators*

μCT was employed to evaluate *in-vitro* the thickness and distribution of gutta-percha around the carrier materials. GuttaCore (GC) obturators (Dentsply Tulsa) and HA/PE (experimental) were prepared from the middle third of the obturators. The samples were characterised further by making three-dimensional reconstructions of all the obturators. Two masks were produced to represent the gutta-percha coating and the carriers. The thickness of GP coating around its carrier was calculated in nine slices for each sample. Four identical fixed points on each slice were identified for thickness measurement in both samples types (GC and HA/PE cores).

2.3.12.2 *Micro-CT evaluation of the percentage of voids within the root canal space of teeth obturated using carrier-based obturation systems*

Volumetric measurements of the root canal obturation were quantified and qualified using μCT. The overall percentage of voids within the obturated root canals was determined with IP software of μCT. The carrier-based obturation system including carrier material and gutta-percha coating with root canal sealer were engaged in the μCT assessment for void detection. The total percentage of voids was estimated by calculating volume of the root canal space and the volume of the root obturation material itself.

2.3.13 Push-out bond strength evaluation of gutta-percha bonded to the carrier materials

In carrier-based obturation systems, adhesion between the carrier material and gutta-percha coating is a clinically relevant issue that may help avoid stripping of gutta-percha coating from the carrier, creating a root canal obturation with fewer voids. Push-out bond strength was used to measure the bond of the gutta-percha

to the carrier material as a compressive force in apico-coronal direction to the carrier material was applied to dislocate the carrier material from the gutta-percha. Ten carriers size #30/0.04 with length of 25mm carriers were selected. The endodontic obturators used in this study were the experimental HA/PE, Thermafil® and GuttaCore.

Chapter 3 Development & characterisation of poly(lactide)/poly(hydroxybutyrate)-hydroxyapatite composite as core carriers for carrier-based root canal obturation for endodontic treatment

3.1 Introduction

Biodegradable polymers sourced from renewable sources are of great interest because they can reduce the environmental burden of waste generated via successful recycling and lowering pollution. Polymers from renewable sources can be a) natural polymers such as starch, cellulose, proteins, b) synthetic polymers from naturally occurring monomers such as lactic acid or c) from microbial sources such as poly(hydroxybutyrate). Aliphatic polyesters such as poly(lactic acid), poly(glycolic acid) or copolymers thereof have been widely used as degradable sutures, orthopaedic devices, scaffolds for tissue engineering and dental applications, whilst poly(hydroxyalkanoates) (PHA) have been considered as a biopolymer family that significantly extend the range of biomaterials suited for biomedical applications (Chen & Wu 2005, Wang *et al.* 2005).

PHB and its copolymers have been shown to improve mechanical properties in polymer blends, whilst exhibiting good cytocompatibility, however they are quite brittle. Polylactic acid on the other hand is a polymer that exhibits a faster rate of degradation by virtue of its structure and currently finds wide application in degradable medical devices. The rationale for using biodegradable polymers in this study is to facilitate their removal by using solvents in cases of retreatment and post space preparation. These groups of polymers are predominantly degraded via a hydrolytic mechanism hence these polymers do not degrade significantly in the absence of water. The polyesters employed in this study were

blends of PLA and PHB as the polymer matrices for forming the composite. Although PHB offers specific benefits of biodegradability, biocompatibility, and thermoplasticity, the high brittleness and rigidity limits its application in areas that need a flexible material (i.e. root canal space) to conform to the irregularity of the intended area. For this reason, PHB is often mixed with other polymers in order to modify the physical and mechanical properties and broadening the utilisation fields (Qiu *et al.* 2003).

PLA was also used in this study as a matrix phase for composite materials. Due to its biodegradability and suitable thermo-mechanical properties, polylactic acid is widely used in medical fields (Urayama *et al.* 2003). PLA is a biocompatible polymer with the eventual degradation product lactic acid that already exists in the human body and can be eliminated by a natural pathway (Schakenraad *et al.* 1988). Polylactic acid exists in two forms; poly (L-lactic) acid which is generated by polymerization of L-isomers and poly (D-lactic acid) which is produced from D-isomers. This means that polylactic acid can be manufactured with a range of characteristics from amorphous to crystalline. Processibility of PLA is of high relevance and allows the fabrication of composite materials in combination with fillers of different shapes such as filaments, rods and fibres. A previous study showed that PLA acid can be driven as a matrix for ceramic particles or adjuvant to calcium phosphate cements and may also be introduced as a carrier for drug delivery (Schnieders *et al.* 2006, Habraken *et al.* 2007, Mourino *et al.* 2013).

It is believed that PLA/PHB polyester blending helps improve the physico-mechanical properties and extends the degradation time by producing new materials tailored for different applications (Malinova & Brozek 2011, Zhang & Thomas 2011). Thus, in this present study, blends of PLA and PHB were prepared in different ratios using a single screw extruder fitted with appropriate

die. The challenging point in root canal re-treatment is the removal a root canal obturation material safely and conservatively. The rationale for employing biodegradable polyesters for endodontic use was therefore based on the fact that self-degradation of the biodegradable material is extremely slow in a closed and dark environment such as the root canal space, whilst, such PHB/PLA biodegradable materials can be completely dissolved in an alkaline medium or solvent when required. Thus blending the two polymers can lead to polymer blends with different physical and mechanical properties.

The reinforcement of polymers using fillers is common in the production and processing of polymeric materials to confer desirable properties. Synthetic and natural hydroxyapatites (HA) have similar chemical composition to the mineral component of bone and teeth. The incorporation of HA into biomaterials for composite formation has been reported to yield composites with good biocompatibility and osteoconductivity to suit their application in bone replacement. The mechanical properties can also be tailored to suit the intended application. Thus, in the present study HA was selected as the filler for forming the composites.

3.2 Materials and Methods

The fabrication procedure of composite fibres was described in Section 2.2.1 (chapter 2). PLA, PHB and their blends with and without hydroxyapatite filler particles were blended in requisite amounts and extruded using a single screw extruder. Different ratios of PLA and PHB were mixed with filler and optimised to function as core carriers for delivering gutta-percha into the root canal space. The newly designed carriers were then successfully coated with gutta-percha in a specific technique described in Section 2.2.1 (chapter 2). FTIR, DSC, DMA, and

Tensile strength were measured to evaluate the suitability of the specifically designed material for endodontic treatment. Five samples were tested in each experimental work.

3.3 Results

3.3.1 FTIR analysis

The FTIR spectra of PHB, PLLA and their composites are shown in Figure 3.1; the FTIR spectral wave range was 4000-650 cm^{-1} . PLLA and PHB are considered to be semi-crystalline polymers and their spectral variations are highly influenced by their corresponding physical states and crystalline structures. In the spectrum of PHB, the band at 1720 cm^{-1} is a sharp strong peak, assigned to the stretching vibrations of carbonyl group C=O, the bands at range of 1300-1200 cm^{-1} particularly at 1291, 1279, and 1263 cm^{-1} were attributed to the stretching vibrations of C-O-C groups and at 1228 cm^{-1} was related to CH₂ group. Further peaks observed in the 1000-800 cm^{-1} region were due to the C-C stretching mode. The PLLA spectrum exhibits a strong peak at 1748 cm^{-1} assigned to the stretching vibrations of C=O, the bands in the 1300-1200 cm^{-1} region attributed to stretching modes of C-O-C and CH₂ groups, also the peaks at 1000-800 cm^{-1} region ascribed to stretching vibrations of C-C, due to the aliphatic methylene groups. In the spectrum of PHB/PLLA with HA composite, the bands at the range 1800-1700 cm^{-1} , 1300-1200 cm^{-1} , and 1000-800 cm^{-1} were examined to detect any interaction that might have occurred in the PHB/PLLA blends. Two peaks, one broad and other one sharp, observed at 1748 cm^{-1} and 1720 cm^{-1} , which were attributed to the C=O stretching modes of PLLA and PHB, respectively indicating the presence of the individual components in the blend. The proximity of the two absorptions lead to the formation of a shoulder rather than two

individual peaks. Anastomosing peaks of both PHB and PLLA are also found in the 1300-1200 cm^{-1} and 1000-800 cm^{-1} regions, suggesting a possible molecular interaction, which needs further investigation.

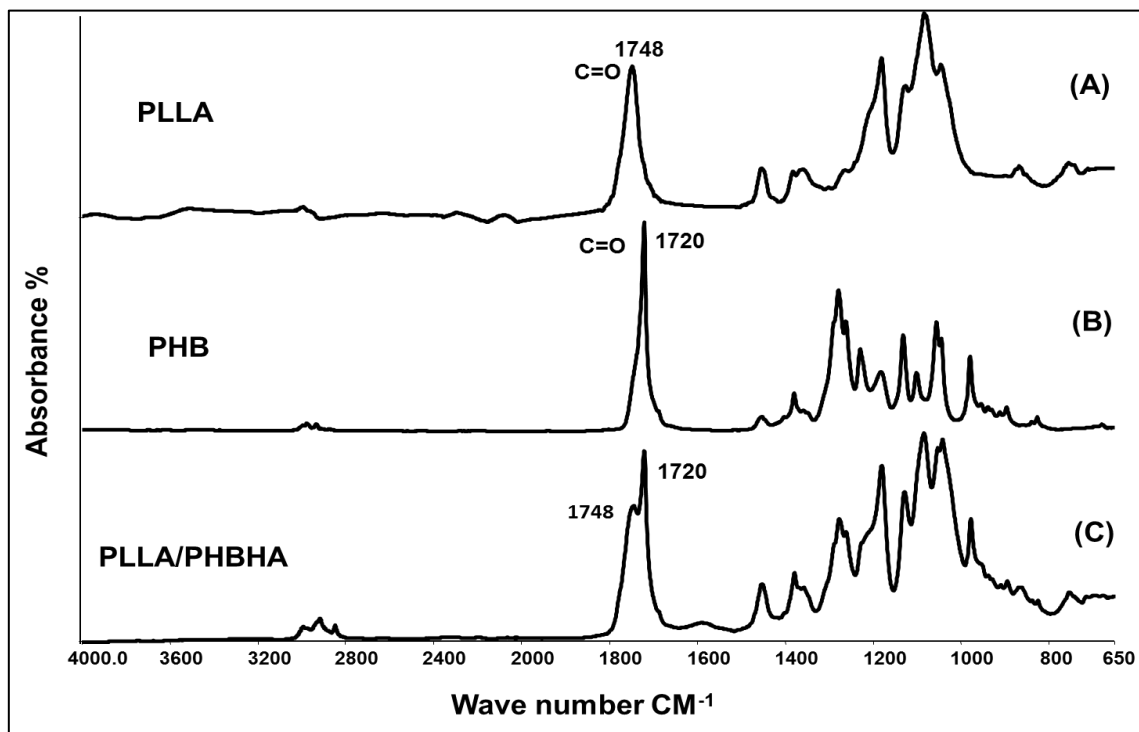


Figure 3.1: FTIR spectra of PLLA (a), PHB (b), and PHB/PLLA blends with HA composite (c)

3.3.2 Tensile test (tensile strength and elastic modulus)

The composition of the blends and composites formed using PLLA, PHB with hydroxyapatite is reported in Table 2.1 (chapter 2), with the tensile strength and modulus of the same formulations represented in Figure 3.2 & 3.3. The tensile strength of the pure PLLA and PHB fibres post-extrusion shows that PLLA exhibits a statistically significant higher value which can be attributed to both the crystallinity and molecular weight. The blending of PLLA with PHB improves the tensile strength as expected and 80% by weight of PLLA in the blend resulted in a strength close to that of pure PLLA. As expected, there was a clear relationship between the amounts of the blending components and the mechanical properties.

The incorporation of PHB may lower the strength and modulus but it had a very slow rate of resorption and thus any effect of residual moisture within the cavity on the degradation is expected to be minimized.

The composites with polymer blends were formed using two concentrations 10 & 20 wt% of the hydroxyapatite as filler. The inclusion of any higher amounts of HA led to limited extrudability in the single screw extruder. The data clearly (Figure 3.3) showed that the HA had an impact on the elastic modulus of the composites and were seen to significantly increase in comparison to the unfilled blends. The data obtained were statistically analysed with one-way analysis of variance (ANOVA) in combination with Tukey's multiple comparison test, the level of significance was set at $P=0.05$. The elastic modulus values of composites containing hydroxyapatite were significantly higher ($P<0.05$) than unfilled non-hydroxyapatite containing PHB/PLLA blends whilst, there was no significant difference ($P>0.05$) between PHB20/HA10 and PHB20/HA20 composites.

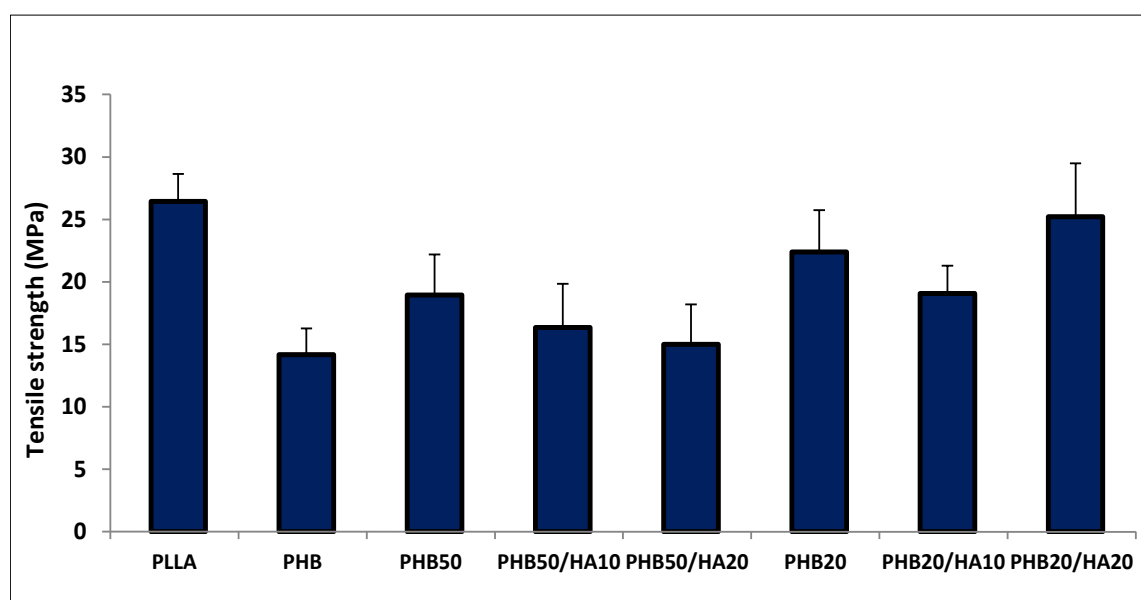


Figure 3.2: Tensile strength of the fibres after extrusion of PHB, PLLA and their blends and composites with hydroxyapatite ($n=5$).

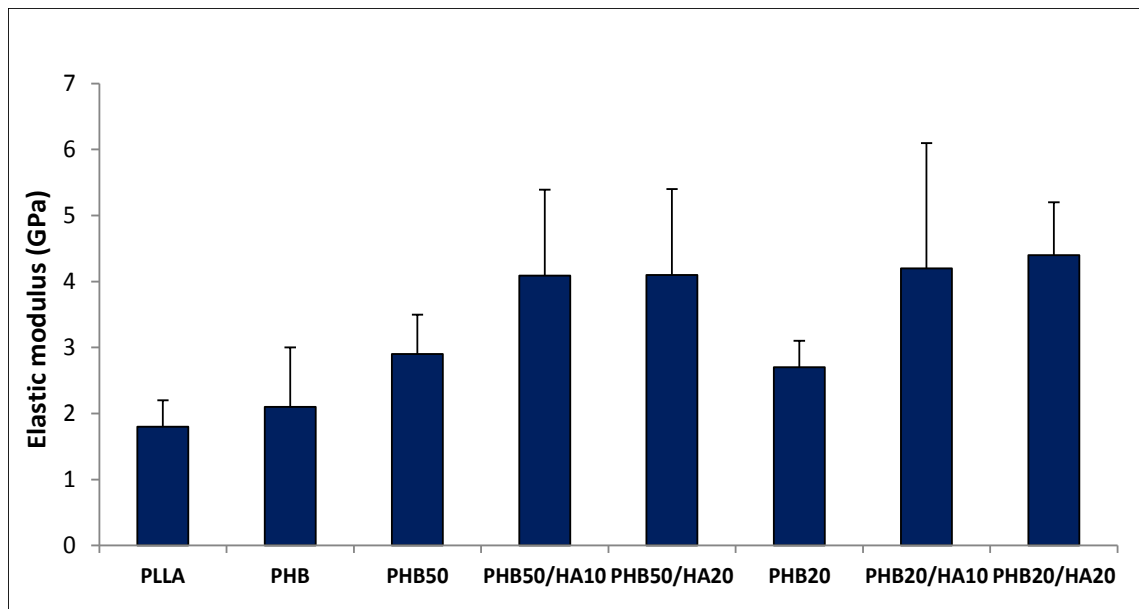


Figure 3.3: Elastic modulus for PHB, PLLA and their blends and composites with hydroxyapatite (n=5).

3.3.3 Thermal properties: Differential Scanning Calorimetry (DSC) and Dynamic Mechanical Analysis (DMA)

3.3.3.1 DSC analysis

The DSC thermograms showed that the glass transition temperature (T_g) of pure PLLA and pure PHB were at 60°C and 7°C, respectively. A remarkable difference in the glass transition temperature between PLLA and PHB was observed, which could help determine the compatibility of blending of PHB/PLLA copolymers at different ratios. PLLA fibres exhibited a melting peak (T_m) at 178°C and a broad cold-crystallisation peak (T_c) around 130°C whereas PHB fibres showed double endothermic peaks at 160°C and 170°C alongside a sharp cold-crystallisation peak around 80°C. Thus, PHB crystallised faster than PLLA, indicating more rigidity than that of PLA. Representative DSC thermograms are shown in Figure 3.4 & Figure 3.5.

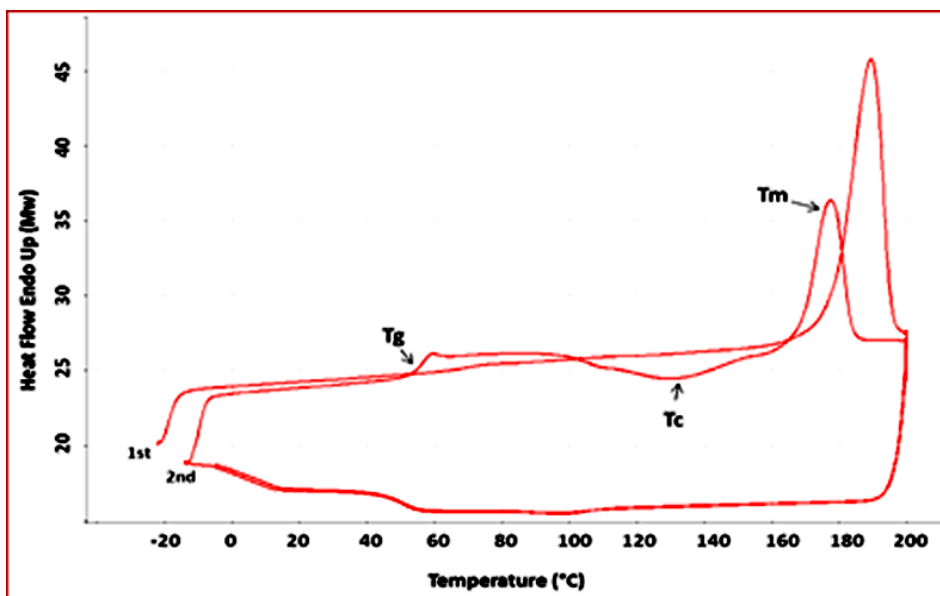


Figure 3.4: DSC thermograms of PLLA fibres, with the two heating and cooling cycles.

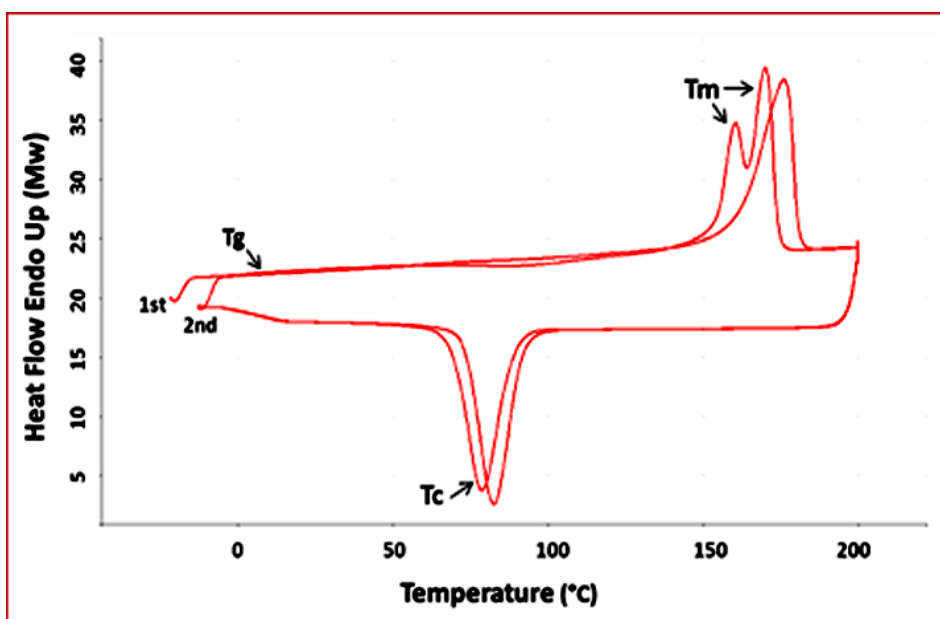


Figure 3.5: DSC thermograms of PHB fibres, with the two heating and cooling cycles.

PHB:PLLA blends were compounded in ratios of (20:80, 40:60, 50:50, 60:40, and 80:20). The T_g of PLLA remained unchanged around 60°C in all the blends whilst the T_c shifted to a lower value than that of neat PLLA, around 108°C-112°C, with the increasing weight fraction of PHB. Apart from the 80:20 ratio, T_c of PLLA in the PHB/PLLA blends ratios were sharper in shape. Triple melting peaks were observed in the DSC analysis of PHB/PLLA in blending ratios of (20:80, 40:60, 50:50, and 60:40) as two of the peaks were attributed to PHB and the third peak to PLLA. However, in the 20:80 blending ratio double melting peaks related to PHB were found at 160°C & 170°C. These DSC findings suggest that the PHB/PLLA blends may be partially compatible. The findings of PHB/PLLA blends obtained with DSC thermograms are reported in Table 3.1 & shown in Figure 3.6.

Table 3.1: Thermal properties of PHB, PLLA and their blends

Polymers	T _g /°C		T _c /°C	T _m /°C
	PHB	PLLA		
PHB	7		80	160 & 170*
20/80		60	112	159, 170 & 178*
40/60		58.5	108	162, 172 & 178*
50/50		59	110	162, 172* & 178
60/40		58.5	110	161, 172* & 178
80/2		60	101	160 & 170*
PLLA		60	130	178

Symbol * represents the strongest peak

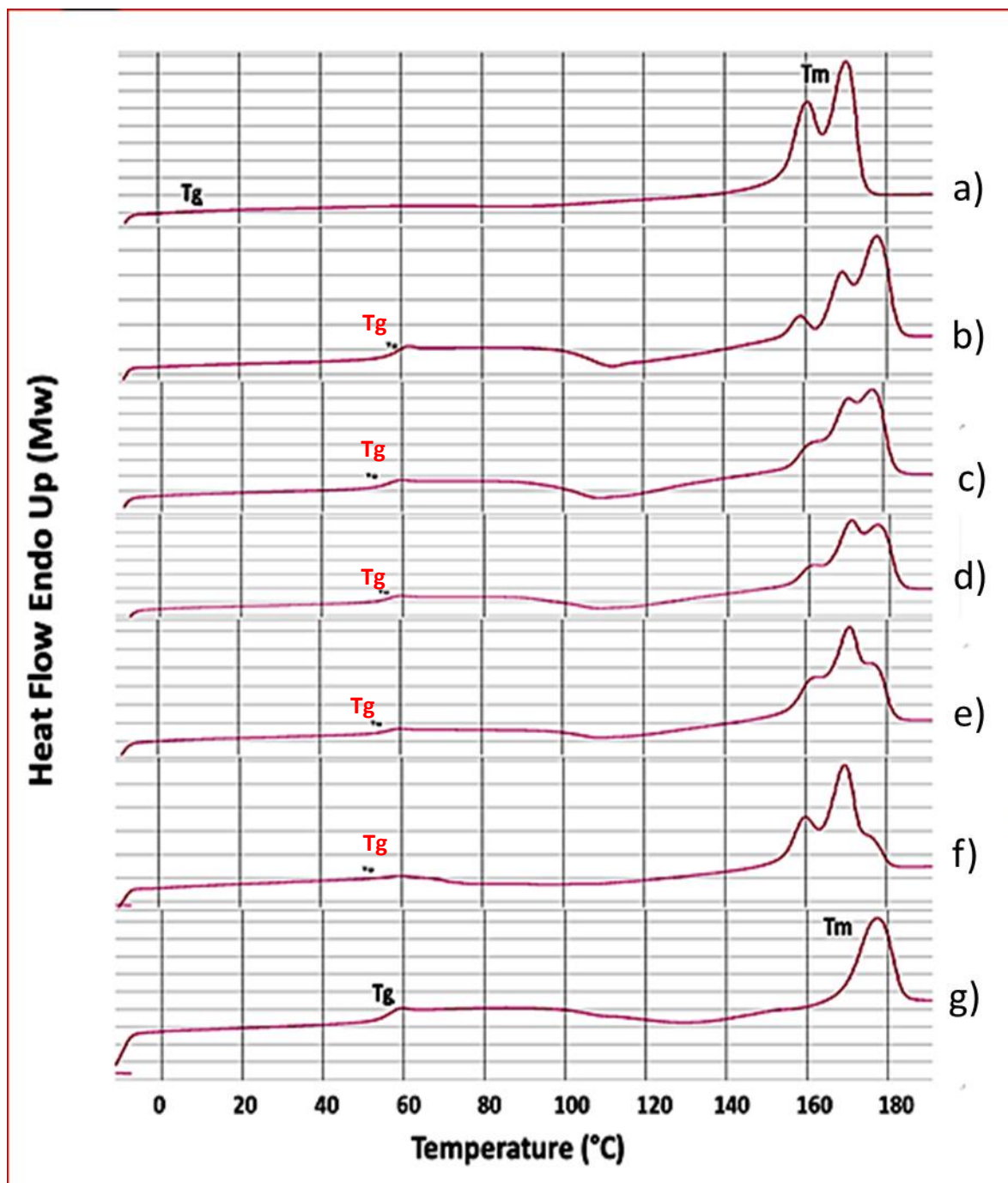


Figure 3.6: Second heating cycle of DSC thermograms indicate transition temperatures of a) neat PHB, b) 20:80, c) 40:60, d) 50:50, e) 60:40, f) 80:20, g) neat PLLA

The incorporation of hydroxyapatite filler into the PHB/PLLA blends showed no significant changes on the glass transition, melting, and crystallisation temperatures as shown in Figure 3.7. On the other hand, the melting point of the commercial plastic carrier of the Therafil[®] system was determined at 350°C, which is remarkably higher than those of the experimental composites.

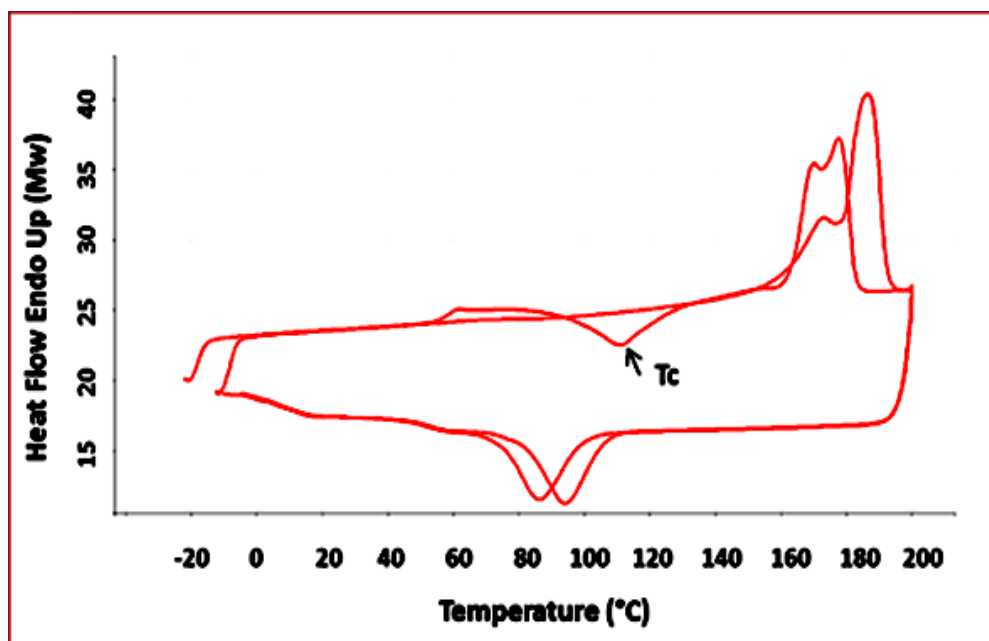


Figure 3.7: DSC thermograms of PHB/PLLA blends with hydroxyapatites, with the two heating and cooling cycles

3.3.3.2 DMA

The results obtained from DMA are reported in Table 3.2 and shown in Figure 3.8. These findings demonstrate that the elastic modulus of the test materials gradually decreases when the temperature increases. Thus, an inverse relationship is noted between the elastic modulus and the temperature reflecting alterations in the material structure. At the onset of glass transition temperature of the material, the elastic modulus dramatically drops as expected due to the segmental motion of the polymer chains that occurs at glass transition. A considerable improvement in the elastic modulus was observed via inclusion of HA fillers into the polymer matrices leading to an increase in the stiffness of the

material. On the other hand, the current carrier material (control) of the Therafil[®] obturator showed a high storage modulus with a glass transition temperature determined at 111°C as shown in Figure 3.9, indicating a certain rigidity compared with the experimental materials.

Table 3.2: Storage moduli, loss moduli and glass transition temperatures (T_g) of the experimental and control materials

Composition	Storage modulus (Pa) at (25°C)	Loss Modulus (Pa) at (25°C)	T _g /°C
Poly-L lactic acid (PLLA)	8.98×10^8	5.14×10^7	68.7
Polyhydroxybutrate (PHB)	4.68×10^8	2.90×10^7	
PHB 50%+ PLLA 50% (PHB50)	3.33×10^8	1.10×10^7	64.1
PHB 50%+ PLLA 50%+ HA 20% (PHB50HA20)	8.11×10^8	3.51×10^7	61.7
Therafil [®] carrier (control)	5.61×10^9	3.93×10^8	111

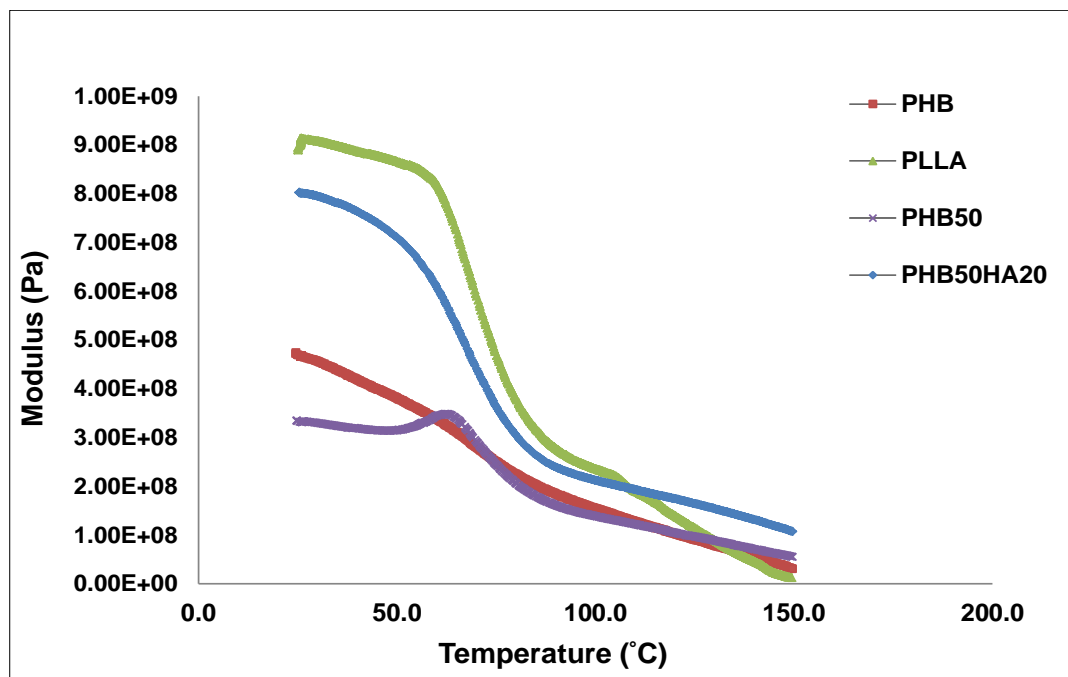


Figure 3.8: Storage and loss moduli of PHB, PLLA, their blends and composite

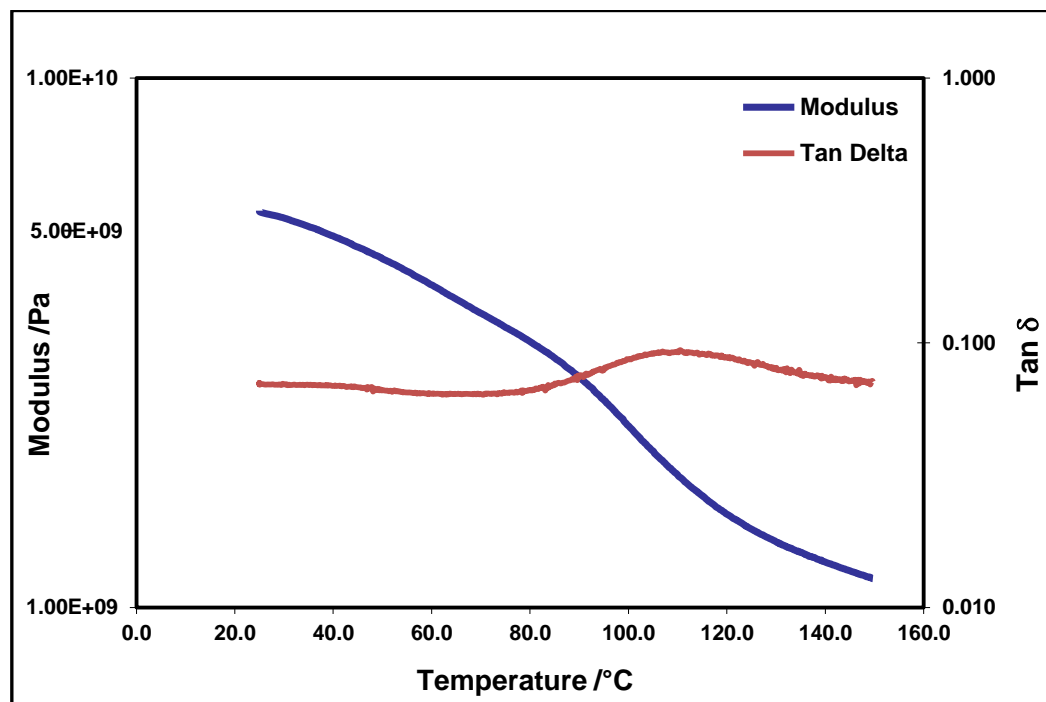


Figure 3.9: Storage and loss moduli of plastic carrier of the Therafil® obturator with the tan delta

3.3.4 Degradation behaviour of the PHB/PLLA-HA composite

The interaction of PHB/PLLA-HA composite was characterised both in simulated body fluid and Hypo-cal[®] (Ellman). The weight changes were determined by immersion in SBF at 37°C to simulate accelerated conditions as the root canal would not be usually exposed to fluids. The interaction test of the composite in Hypo-cal[®] (Ellman) was undertaken to determine the feasibility of removal if required by introduction of Hypo-cal[®] (Ellman) in the root canal.

3.3.4.1 *Interaction of the composite fibres with Simulated Body Fluid (SBF)*

The water uptake profile is shown in Figure 3.10, which clearly indicates that the weight changes are minimal with no significant changes over the evaluation time over 180 days. The only notable changes in the weight of the fibres were observed in the first 24 hours, which may be related to early diffusion. The fibres remained intact as they could be easily picked up with a pair of tweezers.

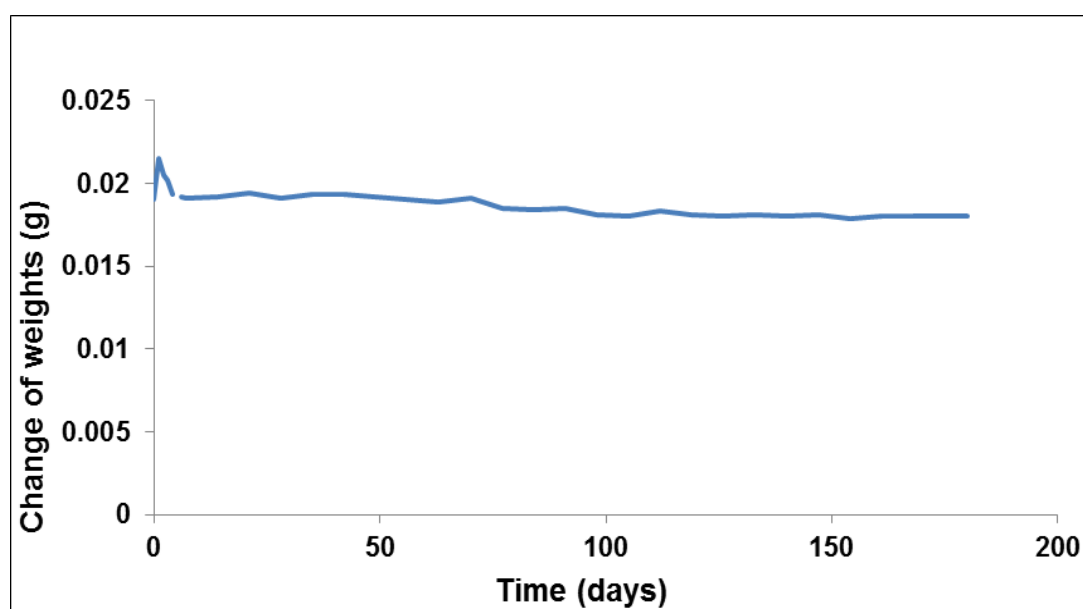


Figure 3.10: A plot of weight (g) against time (days) of PHB50HA20 fibres in SBF.

3.3.4.2 Interaction of the composite fibres with hypo-cal[®] (non-setting calcium hydroxide paste)

Hypo-cal[®] (Ellman) is a popular non-setting calcium hydroxide paste used as an intra-canal medicament. The interaction of the composite fibres with alkaline Hypo-cal[®] (Ellman) was carried out to observe the effect and evaluate the feasibility of removal of the fibres. The polyester-based composite fabricated in this study were completely degraded in alkaline media indicating that the fibres could be removed easily and safely when required. There was a remarkable decrease in the weight of the PHB50HA20 samples from 0.0186 to 0.0069 after two weeks as shown in Figure 3.11. They became a brittle in nature making difficulty to be picked up with a pair of tweezers. A separate study showed that the fibres when placed in the calcium hydroxide paste could be removed intact after 24 hours of contact, which suggested that the carrier could be easily removed and this observation could be explained due to the surface interaction of the alkaline paste and the fibre.

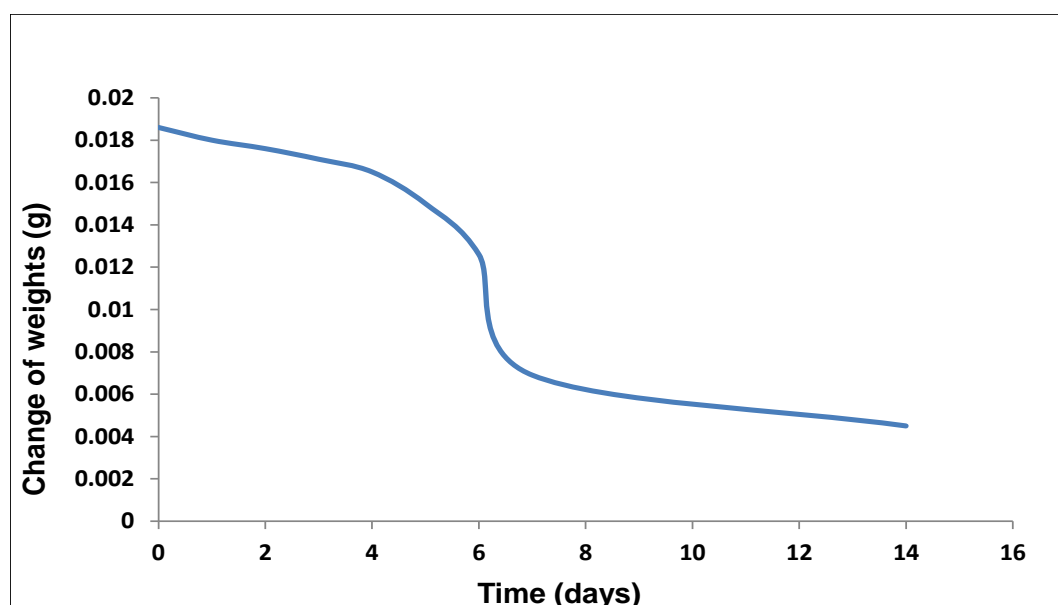


Figure 3.11: A plot of weight (g) against time (days) of PHB50HA20 fibres in Hypo-cal[®]

3.3.5 Biocompatibility

3.3.5.1 MTT assay

The results obtained from the MTT assay established that PHB50HA20 composites exhibited no deleterious effects on the metabolic activity of cells detected at 24 h and at 72 h compared to the negative non-toxic control group as shown in Figure 3.12 & Figure 3.13. The chemical compositions of the PHB50HA20 composite did not release any toxic components in the eluants. At 1-day exposure to eluants from the experimental composite showed a drop in the metabolic activity determined only at 72-h as show in Figure 3.12. Following a 3-day exposure, improvement in the cell metabolic activity was observed at 24-h and 72-h elution as see in Figure 3.13. Thus, MTT assay test indicated a favourable cell-material response with no cytotoxic effect.

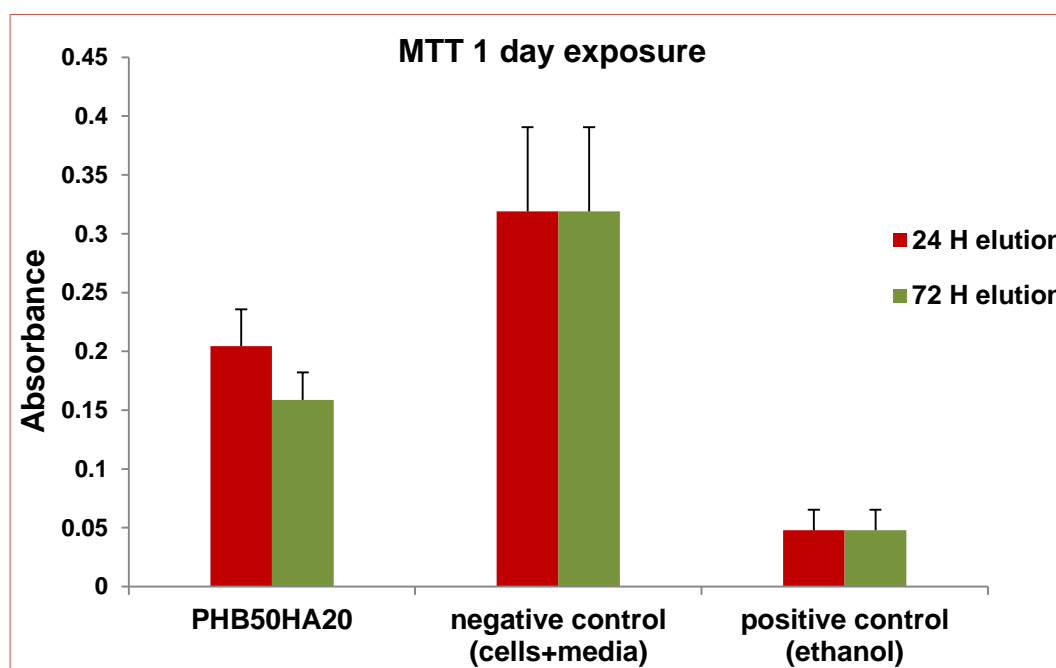


Figure 3.12: Viability of human osteoblast-like (HOB) cells following exposure for 1 day detected at 24-h and 72-h eluted media from the PHB50HA20, expressed as mean & SD absorbance.

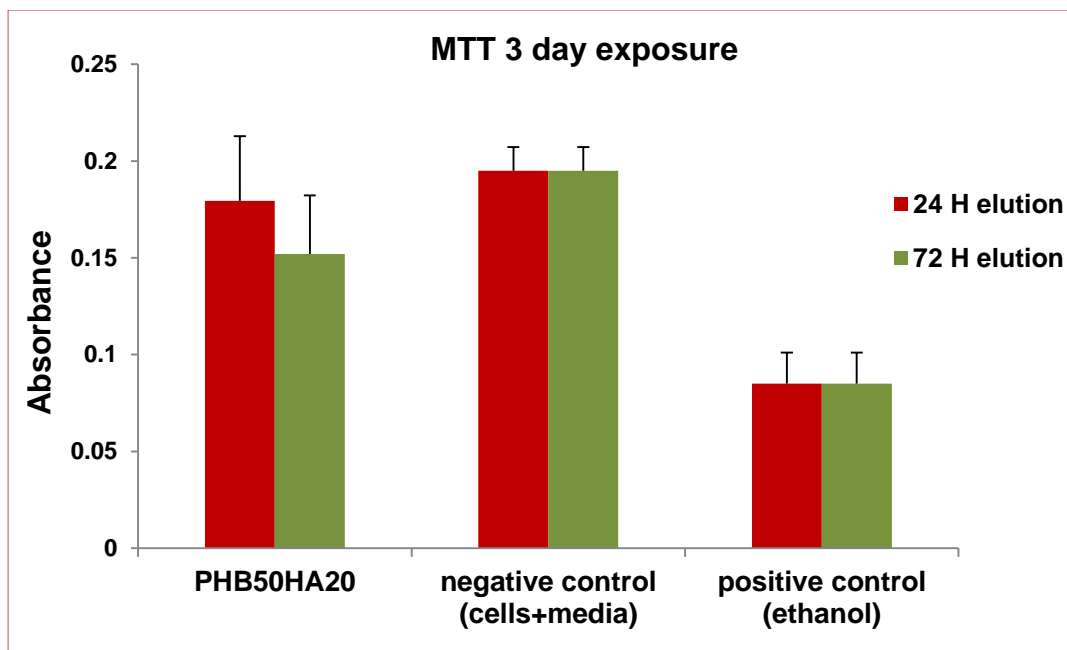


Figure 3.13: Viability of human osteoblast-like (HOB) cells following exposure for 3 day detected at 24-h and 72-h eluted media from the PHB50HA20, expressed as mean & SD absorbance.

3.3.5.2 *alamarBlueTM assay*

The results of the cell proliferation study (alamarBlueTM direct assay) on the test materials detected at 1, 3, 7, 14, 21, and 28 days compared with ThermanoxTM (TMX) (tissue culture control surface), are shown in Figure 3.14. It is apparent from alamarBlueTM assay that cell proliferation on the PHB50HA20 composites showed no significant difference ($P > 0.05$) in comparison to the non-toxic control ThermanoxTM at all-time intervals, suggesting that the PHB/PLLA-HA is biocompatible material. No increase in the cell proliferation was noticed for the time periods investigated on the TMX. Thus the test material showed a similar trend in proliferation.

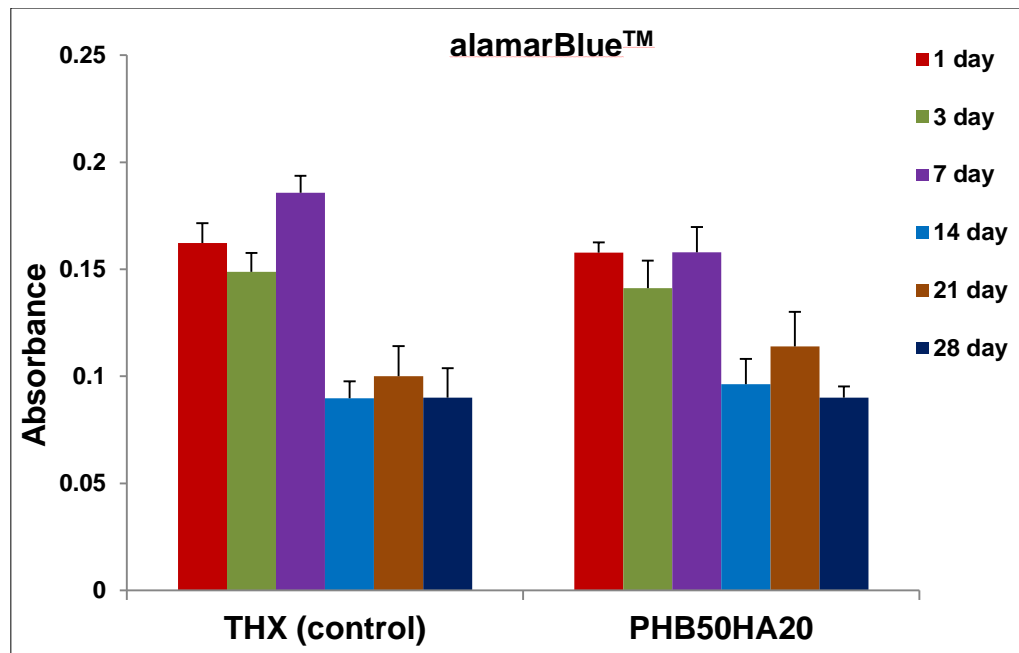


Figure 3.14: Proliferation of human osteoblast-like cells as indicated by alamarBlue™ assay in direct contact with the PHB50HA20 composite at 1, 3, 7, 14, 21 and 28 days.

3.3.6 X-ray contrast

The results obtained from the radiographs analysed using image J software showed a mean density of 90 (grey scale value) for the polyhydroxybutrate 50/50 polylactic acid with hydroxyapatite 20%, (PHB50HA20) composite placed in the aluminium washer, which is equivalent to 3.2 mm Al when compared with steps of aluminium step wedge as shown in Figure 3.15. The radiograph of the experimental composite seems to be radiopaque but some improvement in the radio-contrast media is required as it is not likely to be easily discriminated from the dentine of the root canal.

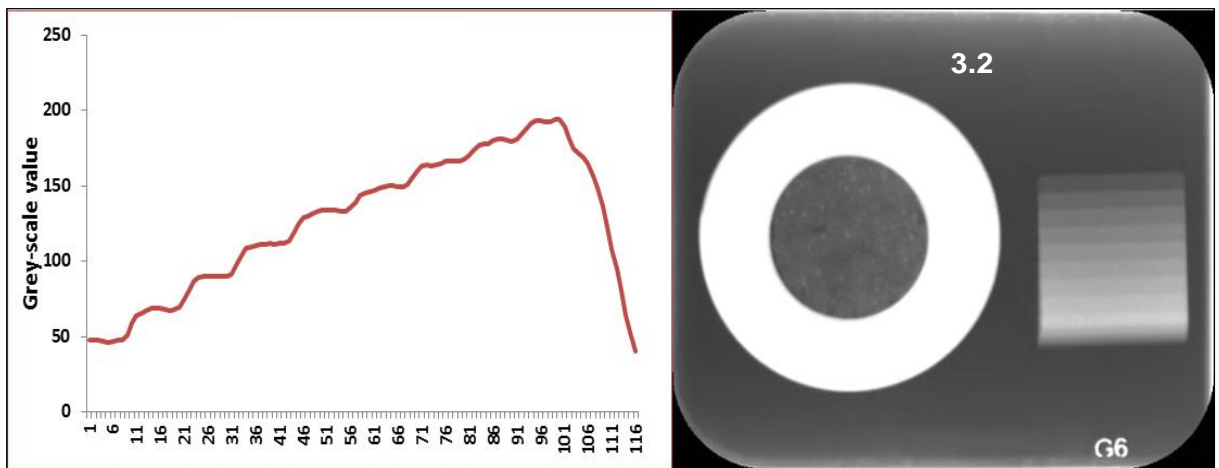


Figure 3.15: Radiograph shows the radiopacity of the PHB50HA20 composite in the washer estimated in relation to the density of the aluminium steps wedge using image J software plot profile analysis (n=5).

3.3.7 Technical procedure required for removal of polyester-based composite core carriers from the root canal in retreatment or post space preparation cases.

The polyester-based composite used as a core-carrier is simple to retrieve, and the retrieval can be achieved following two steps:

i) Gutta-percha removal

The gutta-percha adhering to the carrier can be removed either by using solvents such as chloroform or using hand and rotary files, heat-carrying instrument or even ultrasonic tips. In the present study, a heat-carrying instrument was used to help removing the coronal third of the gutta-percha and exposing the carrier.

ii) Carrier material retrieval

Injection of hypo-cal[®] into the root canal space allows the dissolution of the exposed carrier and allows the insertion of a hedstrom file that would help removing completely the carrier.

3.4 Discussion

The prevention of ingress of bacteria in the radicular space prepared for root canal therapy is vital for the success of endodontic therapy. Hence, if obturating materials and techniques can be improved to prevent coronal leakage and reinfection, it is expected to minimize the need for re-treatment. Gutta-percha continues to remain the most commonly employed root canal obturation material, followed by Resilon[™], which is fast gaining acceptance. Although the obturating material is important, the obturation technique has an important role to play and of the different techniques used, carrier based obturation is one such method. Thermafil[®], a commercial material that is generally used as a carrier for gutta-percha, the advantage of the carrier-based obturation system being the ability to fill the root canal space in three dimensions. However, current carrier materials are not easy to retrieve and their handling properties in terms of elasticity are still inadequate in case of severe curvatures.

Clinical experience clearly indicates that not all off-the-shelf materials are suitable for biomedical applications and thus have to be clearly formulated to suit a particular clinical application and the biocompatibility of the device needs to be evaluated. Composite formulation can lead to versatile properties and PHB and PLA were used in the current study to form the matrix of the composite for developing carrier based materials. PHB and PLLA are thermoplastic in nature, biocompatible and their thermal properties make them suitable for melt extrusion. PLLA is a biodegradable polymer that has been widely used in several biomedical applications (Eligio *et al.* 1999) with degradation products that are non-toxic (Blümm & Owen 1995) and eliminated from the body by natural pathways. PHB, is a biocompatible polymer, which has found application in biomedical applications, however it has a much slower rate of degradation and

different physical properties with much higher hydrophobicity. In addition, the retrievability of the polyesters that can be facilitated by using alkaline mediated hydrolysis in cases of treatment failure. Hydroxyapatite was selected as the filler for the composite, which is a naturally occurring mineral form of calcium apatite and has been extensively used in fabricating materials for bone substitution. Thus, fibres of pure PLA, PHB and their blends with or without HA filler were developed, for use as core carriers.

A single-screw extruder device with high shear forces was employed in the current study to fabricate homogenous fibres. Melt extrusion of the polymer blends was possible due to the low melting temperatures of the PLLA-PHB blends. A study was conducted to determine miscibility of the two components and a range of concentrations were first extruded and based on the thermal properties, a 50:50 and 20:80, PHB:PLLA were used to develop the composites.

The experimental PLLA, PHB fibres, their blends and composites were characterised using FTIR. It is apparent from FTIR spectra that the absorption band at 1748 cm^{-1} , is attributed to carbonyl stretching, (C=O) of PLLA and the absorption band at 1720 cm^{-1} , is assigned to carbonyl stretching, (C=O) of PHB. A peak that emerged at 960 cm^{-1} is attributed to the phosphate group (P-O) of HA filler. The spectral analyses were in agreement with previous studies and as expected for absorption peaks of ester carbonyl, phosphate and methylene carbons (Zhang & Thomas 2011). Furthermore, the peak intensity at 1720 cm^{-1} of PHB appeared sharper in comparison to the peak at 1748 cm^{-1} of PLLA, suggesting that PHB is a highly rigid and crystalline material.

The tensile strength was determined for the pure polymers, the PHB:PLLA blends and the composites using same specimen dimensions. The tensile strength of

PLLA was found to be significantly higher than PHB, which can be attributed to the crystalline nature of the PLLA polymer. Although PHB is crystalline, the low nucleation density of PHB results in large spherulites with cracks and splits, rendering it brittle. Thus an increasing concentration of PHB in the physical blends showed a lowering in the tensile strength and with the addition of the hydroxyapatite filler an increase was observed due to the presence of the particulate filler; however it remained lower than PLLA. The crystalline arrangement of PLLA is disrupted in the blends and with the filler particles in the composite. However, as expected the modulus of the composites increased with the content of PHB and HA in the blends and composites respectively. The mechanical tests showed that the PHB/PLA-HA composite fibres exhibited high stiffness. It is difficult to compare these results with literature findings since most composites of PHB-HA or PLLA-HA report data in compression and are inevitably tested in a porous form, since they are proposed as scaffolds for bone tissue engineering. The results established that the addition of PLA to PHB improved the mechanical properties of PHB (tensile strength) whereas tensile strength of PLA decreased and an increase elastic modulus was observed in the PHB/PLLA blends which indicated that PHB crystallizes before PLA. This finding may be attributed to good dispersion of PHB in PLA acting as filler. Similar findings have been reported by previous study (Zhang & Thomas 2011), wherein the preparation and characterisation of PHB/PLA blends have been investigated and thus they showed improvement in the mechanical properties of PHB by blending with PLA. However, the high stiffness of the fibre composites limits the efficacy of these to be clinically applied as a carrier material.

DMA has been proven useful to evaluate the viscoelastic properties of polymers. It was assumed that the physiological frequency was 1 Hz; hence all the

measurements were made at this frequency. The storage modulus was obtained in the temperature range of 20–150°C for the polymers, blends and composites. The results of DMA demonstrated that the storage modulus and loss modulus of elasticity of PLA are higher than those of PHB and their blends and also showed an increase in the storage modulus by addition of HA fillers whereas the damping property (tan delta) reduced with an increase in the amount of HA filler. As expected, the storage modulus increased with the presence of the bioceramic filler and decreased with the increase in temperature. It has been shown in a previous study that the storage modulus increased and damping decreased with the addition of hydroxyapatite to PLA (Nazhat *et al.* 2001), wherein the HA reinforced biodegradable PLA had been formed for the repair and augmentation of bone.

The thermal properties of PHB, PLLA and their blends and composite were characterised using DSC. The identification of the melting temperatures is an important parameter to ensure that no thermal degradation of the polymers occur. Thus, initial determination of melting temperatures was investigated via DSC. The thermograms shown in Figures 3.6 & 3.7 showed that the glass transition temperature of neat PHB and PLLA were 7°C and 60°C respectively. A similar finding was reported by previous study (Furukawa *et al.* 2007), wherein the structure and miscibility of PHB/PLLA blends were characterised using several tests including DSC to determine the glass transition, melting, and crystallisation temperatures. PHB, belongs to a class of polyhydroxyalkanoates, which is highly crystalline and is reported to have a melting temperature of around 180°C. The thermogram of PHB showed the appearance of a double melting peak (Figure 3.6). The lower melting peak is often attributed to the existing PHB crystals whilst the higher peak is exhibited due to the crystals formed by recrystallization of the

PHB during the heating cycle of the DSC run. One of the problems associated with processing of PHB is its thermal instability and chain scission may occur whilst processing at its melt temperature, thus forming a blend with PLLA is expected to reduce the risk of degradation during melt extrusion. The miscibility of the PHB/PLLA blends may be spotted across identifying the number of phase separation of DSC thermogram (Qiu *et al.* 2003, Malinova & Brozek 2011). DSC results of PHB/PLLA blends in ratios of 50/50 and 20/80 showed one value of T_g at 58.5-60, indicating that the blends were partially compatible. The addition of HA filler showed no significant effect on the thermal properties of blends. The crystallization rate of PHB was remarkably higher than that of PLA which was observed in the DSC scans. The addition of PLA in different ratios therefore improved the thermal stability of the blend. Although the crystallinity was decreased, the polyester-based composite fibre showed high stiffness that limits its application in endodontics.

The degradation behaviour of the composites was evaluated both in simulated body fluid and calcium hydroxide. Hypo-cal[®], a calcium hydroxide paste, is a commonly used medicament in the canal to reduce residual microbial flora. Calcium hydroxide has the ability to exert lethal effects on bacteria (Bystrom *et al.* 1985, Stuart *et al.* 1991), thus is an effective method of minimizing bacterial population in the root canal. The interaction of the composite fibres with Hypo-cal[®] resulted in complete dissolution of the fibres in two weeks, which is probably due to the alkali mediated hydrolysis. However, the fibres were able to be removed intact once they had interacted with the calcium hydroxide paste for 24 hours, suggesting that the removal of these fibres was easy and clinically feasible as opposed to Thermafil[®], which requires a very high temperature for its removal. Furthermore, the degradation behaviour of the polyester-based composite fibres

when immersed in SBF at 37°C showed no evidence of degradation of the materials over 6 months. This aggressive testing environment wherein the fibres are actually placed at 37°C in the immersion medium is probably comparable to many years within a root canal since moisture penetration into a well obturated root canal is likely to be very small; hence the risk of composite degradation in such an environment is expected to be minimal.

Biocompatibility of any clinical medical device needs to be evaluated in the final form of its clinical use. Thus the composites that were extruded were shaped in the form of discs to assist testing whilst maintaining the same parameters used for extrusion of the fibres. *In vitro* cell culture studies using alveolar human osteoblast like cells, represented by MTT and alamarBlue™ assays were undertaken to characterise the cytocompatibility of the experimental PHB/PLLA-HA composite. At 1 day cell exposure of MTT assay, the cell viability and metabolic activity showed significant changes in comparison to the control. Whereas at 3 day cell exposure of MTT, an improvement in the viability and metabolic function was observed in particular at 72-h elution and thus, it is apparent from MTT results that no leachable toxic ion release. The alamarBlue™ assay was also conducted to characterise the cell proliferation. The results obtained from alamarBlue™ assay demonstrated that the cell metabolic activity and proliferation on the polyester-based composites exhibited no significant difference in comparison to the non-toxic control Thermanox™ over 28 days and a slight enhancement of cell proliferation was observed at 14 days. This enhancement may be related to the presence of hydroxyapatite which provides favourable conditions for cellular adhesion and attachment.

Radiopacity is another prerequisite for any root canal obturation material that it is important for diagnostic and treatment evaluations. Thus, the radiopacity of the

polyester-based composites were assessed with digital radiography and a storage-phosphor system. The radiographic images were analysed using image J software. In accordance with the standards established by ANSI/ADA (2000) Specification No. 57, root canal obturation materials should possess a radiopacity not less than that equivalent to 3 mm of aluminium. It is apparent from the results of the radiographs that the polyester-based composite exhibited a requisite radiopacity, around 3.2 mm Al equivalent, to function as a carrier for carrier-based obturation systems, however this radiopacity is not close enough to the gutta-percha. The radiopacity requires further improvement, however, if more fillers are added to the matrix, the rigidity of the fibres may also be increased.

3.5 Conclusion

Composites of blends of PLLA-PHB with hydroxyapatite filler were fabricated in the form of fibres suited for endodontic carriers. The fibres exhibited adequate mechanical and thermal properties with reliable methods for its easy removal; however the high stiffness of the composite limits its clinical handling, which will require further manipulation for the intended application.

Chapter 4 Development & characterisation of low density polyethylene-hydroxyapatite composites as core carriers for carrier-based root canal obturation for endodontic treatment

4.1 Introduction

This chapter describes the fabrication and characterisation of low density polyethylene hydroxyapatite composites shaped as core carriers for carrier-based root canal obturation. *In vitro* studies including fluid filtration, micro-computed tomography and push-out bond strength are reported that were specifically carried out to assess the suitability of the newly designed carriers for endodontic application. Although carrier-based gutta-percha is considered to be an effective method of root canal obturation creating a 3-dimensional obturation, a suitable carrier is expected to ease placement and minimise dental chair time. In order to design the carriers in this study, low density polyethylene was selected to confer flexibility and hydroxyapatite was used in conjunction with SrO to achieve the radiopacity required for a carrier material, and the quantity of the HA was adjusted to tailor the flexibility of the carriers.

Composites of HA and high density polyethylene (HDPE) have been reported as bone analogue materials (Bonfield 1988) and are currently used as middle ear implants (Tanner 2010), have established biocompatibility and thus are suited for biomedical applications. Furthermore, HA/PE composites can be rendered flexible, tailored to exhibit desirable flow properties (Ngu *et al.* 2009, Madera-Santana *et al.* 2010, Suresh *et al.* 2011), and are cytocompatible (Bonfield & Tanner 1997, Di Silvio *et al.* 2002, Zhang *et al.* 2007, Parra *et al.* 2009). HA/PE composites have potential to be designed as carriers for carrier-based obturation

systems. The relevance of the experimental HA/PE obturator system for root canal obturation was characterised *in vitro* using various tests.

An ideal root canal obturation should achieve a hermetic seal both along the canal walls and in particular in the apical portion of the root canal (Da Silva *et al.* 2002). If these targets are not accomplished, it is likely that leakages and contamination of the root canal space may be encountered causing periapical lesions and/or failure of the endodontic treatment (Magalhaes *et al.* 2007). Several concerns have been raised on the ability of carrier based root canal obturation techniques to seal oval-shaped root canals (Ibarrola *et al.* 1993), and in particular, concerning the complexity in the removal of the carrier during endodontic re-treatments. Indeed, the development of new carrier-based obturators is required in order to facilitate the retreatment procedures in case of failure of the endodontic treatment (Gopikrishna & Parameswaren 2006).

The assessment of the sealing is one of the important aspects in the evaluation of a newly developed obturation system (Nawal *et al.* 2011). The sealing ability measurements can be undertaken by using dye penetration (Kumar & Shruthi 2012), microbial leakage assay (Monticelli *et al.* 2007, Bakhtiar *et al.* 2012) or fluid filtration, which seems to represent more precise and less subjected to individual's qualitative scoring or bacteria-growing related (Sagsen *et al.* 2006, Gandolfi *et al.* 2007, Genc *et al.* 2011). Thus, the fluid filtration technique was conducted in this study to assess the sealing ability of the experimental HA/LDPE obturator and compared with that of GuttaCore (Dentsply) when applied in single-rooted extracted human teeth or simulated root canals. GuttaCore is a recently marketed obturator system consisting of a carrier made from cross linked gutta-percha surrounded by alpha gutta-percha with the aim of facilitating endodontic re-treatment.

Beside the sealing ability assessment, percentage of void volume of the experimental obturator within the root canal system was also characterized using μ CT measurements. Achieving long-term success of endodontic treatment needs adequate obturation of the disinfected root canal space (Epley *et al.* 2006). Obturation of the root canal aims to create a fluid-tight seal with no voids by using appropriate root canal obturation materials (Michaud *et al.* 2008). However, most root canal obturation materials do not thoroughly obturate the root canal system leaving some voids either within the root canal obturation material itself or at their interface with dentine (Kontakiotis *et al.* 2007). These voids might harbour bacteria that can multiply when in contact with nutrients via the periapical region or lateral canals (van der Sluis *et al.* 2005).

Several methods are available to obturate the root canal space. Previous studies have shown the effectiveness of thermoplasticised core carrier techniques in obtaining three dimensional root canal obturation in a reduced amount of time compared to the lateral condensation technique (Becker & Donnelly 1997, Clinton & Van Himel 2001). As described previously, many methods have been reported to assess *in-vitro* the obturation of the root canal, including dye penetration (Wu & Wesselink 1993, Inan *et al.* 2007) complete dissolution of teeth in acid (Brayton *et al.* 1973), SEM observations (Mannocci *et al.* 1998) and microbial leakage (Jacobson *et al.* 2002, Bakhtiar *et al.* 2012). However, these techniques are unable to provide accurate results as they assess the obturation of the root canal space in two-dimensions rather than in three-dimensions (Branstetter & von Fraunhofer 1982).

High resolution micro-computed tomography is an emerging technology used as a research tool in various fields of dentistry (Jung *et al.* 2005, Barletta *et al.* 2007, Hammad *et al.* 2009). In the endodontic field of research, μ CT has been used to

assess the obturation of the root canal system via measurement of the percentage volume of voids at the canal wall-obturation material interface and within the obturation material itself (El-Ma'aïta *et al.* 2012, Zogheib *et al.* 2012, Zogheib *et al.* 2013).

It is generally accepted that the outcome of the root canal treatment is positively correlated with the technical quality of the root canal obturation (Ng *et al.* 2008). Various types of carrier-based obturation systems are currently available on the market including Thermafil[®], Densfil (Dentsply Maillefer), GuttaCore (GuttaCore, Dentsply Tulsa Dental). One potential disadvantage of currently available carrier-based obturation systems is that the volume of gutta-percha is not uniformly distributed around the carrier. Stripping of gutta-percha from the carrier material might occur during placement of the obturator into root canal space, leading to possible voids (Weller *et al.* 1997, Wesselink 2003). The frictional forces present between the gutta-percha and the root canal walls may create an extrusion effect, whereby the obturation material is retained at the orifice of the canal (Bertacci *et al.* 2007).

Previous studies using μ CT have shown the possibility of conducting both volumetric measurements (Hammad *et al.* 2008) and the 3D reconstruction of obturated root canals (Jung *et al.* 2005). This work was undertaken to assess and compare the volume of gutta-percha around the carriers of GuttaCore and HA/LDPE obturation systems. In the present study, μ CT was employed to compare the root canal obturation performed using two carrier-based obturation systems, the experimental HA/PE and GuttaCore.

In addition, the retention of gutta-percha to the carrier of core-based root canal obturation systems is often clinically unsatisfactory. Another potential

disadvantage of a carrier-based root obturation system is denudation of the core (Rapisarda *et al.* 1999). Previous studies demonstrated the most common causes of stripping of the gutta-percha coating and subsequent exposure of the carrier are twisting of the carrier during insertion into the root canal space (Levitan *et al.* 2003) and inadequate amounts of sealer placed prior to insertion of the obturators in the root canal (Buchanan 2009). A sort of adhesion between the carrier material and gutta-percha coating is required to avoid stripping of the gutta-percha coating creating a root canal obturation with fewer voids.

Thus push-out test was performed in the present study to compare the bond strength between gutta-percha coatings and current carrier materials, in particular, Thermafil[®] (Dentsply Tulsa Dental, Tulsa, OK), GuttaCore (Dentsply Tulsa Dental) and, an experimental carrier made of hydroxyapatite-polyethylene (HA/PE) and strontium oxide. The overall objectives of the present study were to fabricate and assess the thermo-mechanical properties of newly designed carriers, for carrier-based root canal obturation, obtained from a low-density PE and HA composite with the inclusion of strontium oxide and to evaluate their radiopacity, sealing ability, and bondability to gutta-percha coating.

4.2 Materials & methods

The materials and methods employed to fabricate the composite fibres as core carriers for delivering gutta-percha into the root canal space are described in detail in Section 2.2.1 (chapter 2). The polymer processing technology used to fabricate HA/PE composites to function as carriers is the same used for the fibres fabricated for the obturation core material and only differ in the concentration and optimisation of the constituents of the composites. *The in-vitro* assessment methods of the experimental HA/PE obturators for root canal obturation are described below:

4.2.1 Fluid filtration technique

Preparation of human and simulated root canals

Thirty single-rooted human extracted teeth and thirty simulated canals were selected for this study with consent of the appropriate ethical institutional review board. Roots with open apices, cracks and perforations were discarded. The teeth were carefully cleaned with curettes to remove soft tissue remnants and were stored in saline solution before instrumentation.

The crown of each tooth was removed 1 mm above the cemento-enamel junction using a low speed diamond wafering blade (Isomet; Buehler, Lake Bluff, IL, USA) and the coronal third of each root was instrumented with Gates burs 2 & 3, (Dentsply) with a low speed handpiece. All the teeth were treated by the same operator. The working length was determined by subtracting 1 mm from the total length of the root by placing a size 15 K-type file (Kerr, Romulus, USA) into each root canal until the tip of the files was visible at the end of the apical foramen. The apical portion of the canal was instrumented to an ISO size 30 master apical file using the step-back technique with K-type files. The root canals were irrigated

using 10 mL of 2.5% sodium hypochlorite (NaOCl) after each step procedure and the smear layer was conditioned using 10 mL of 17% ethylene-diamine-tetraacetic acid (EDTA) (Septodont, Saint-Maur-des-Fossés, France) for 10 minutes, followed by 10 mL of 2.5% NaOCl. Finally, the root canals were flushed with 3 mL saline solution and dried with ISO paper points, size #30.

Experimental design and obturation procedures

The human and simulated root canals were randomly divided into four experimental groups (n=6/each group) and negative and positive control groups. The specimens in the negative group were completely filled and covered with cyanoacrylate glue (Zapit, Dental Ventures of America, Corona, USA), while the positive control specimens were left with no obturation material. All the specimens were coated entirely with two layers of nail varnish. The specimens in the experimental groups were obturated using the GuttaCore carrier-based root canal obturator system (Dentsply Tulsa, Johnson City, USA) and using the experimental HA/PE carrier-based obturator system with or without the use of the Tubliseal (SybroEndo, Orange, CA, USA). A modified Thermafil[®] oven was used to thermoplasticise the experimental HA/PE and control endodontic obturators. The specimens were finally stored in an incubator for 72 hours at 37°C to allow for complete setting of the sealer.

High-pressure hydraulic fluid filtration system and sealing ability evaluation

The specimens were glued (Zapit) to a Perspex[™] (Perspex Distributions, London, UK) platform (2 cm × 2 cm × 0.5 cm) that was perforated by an 18 gauge stainless steel tube. Each specimen was connected to a hydraulic fluid filtration system able to deliver a hydrostatic water pressure via nitrogen gas pressure (10 psi/69 kPa) and polyethylene tubing through the coronal end of the roots. A 25 mL capacity micro-capillary tube (Microcaps, Fisher Scientific, Atlanta, GA, USA)

was positioned above a millimetric ruler and horizontally between the pressure reservoir and the crown segment as shown in Figure 4.1. The linear displacement of an air bubble inside the micro-capillary tube, which indicated the volume displacement, was detected using a high definition digital camera (Sony HDR-XR500V) placed vertically 5 cm above the micro-capillary and connected to a PC monitor via USB cable.

The fluid transudation through the obturated root canal (i.e. sealing ability) was performed according to the hydraulic conductance protocol reported by Tay *et al.* (2007) using a modified fluid filtration system. The fluid filtration of the tested materials applied in human and simulated root canals was evaluated at different time intervals (30, 60 and 90 minutes). Data were plotted as fluid flow (μLmin^{-1}) versus time and expressed as mean fluid flow. Because the normality (Kolmogorov-Smirnoff test) and homoscedasticity (Levene test) assumptions of the data were violated, data were analysed using one-way ANOVA and Dunn's multiple comparison test at $\alpha = 0.05$.

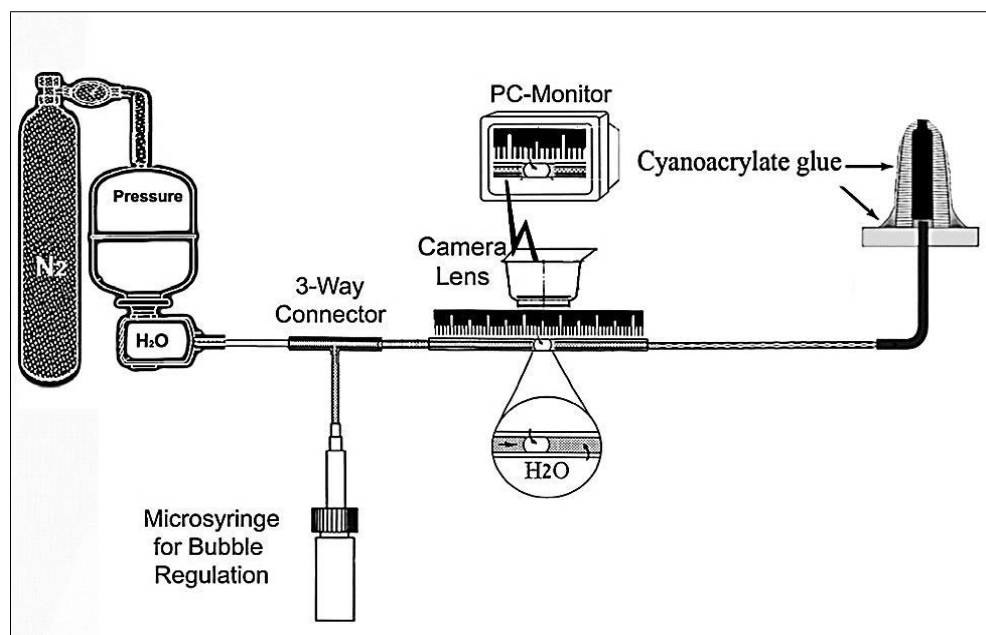


Figure 4.1: Schematic representation of the fluid transport model used in this study.

4.2.2 Micro-Computed Tomography (μ CT)

4.2.2.1 Voids volume qualification within the root canal space

Tooth selection and instrumentation

Eight straight single-rooted human permanent teeth were selected and disinfected by sodium hypochlorite and then stored in sterile water. Teeth with open apices, root caries, root restoration, root defects and root resorption were excluded from the study. A root length of 12 mm was achieved by cutting the crown of tooth with a diamond wheel saw. Access into the canals was performed, and a glide path was created by inserting a hand K-file size # 10 (Dentsply Tulsa Dental, Tulsa, OK, USA) into the canal until it appeared from the apex; this length was calculated and the final working length was fixed at 1 mm short of that length. The cleaning and shaping the root canals was carried out with a crown-down technique using Gates Glidden drills sizes 2, 3 and nickel-titanium hand instruments (Dentsply Maillefer). The apical diameter of the canal was prepared to size # 30/0.04 for achieving standardised measurements. During preparation,

irrigation of the root canals was carried out with 2.5 mL 2.5 % sodium hypochlorite (NaOCl). Then the canals were finally flushed with 5 mL 17% EDTA in order to remove the smear layer followed by 5 mL 2.5 % NaOCl and rinsing with normal saline.

Obturation of the canals

The cleaned and shaped roots were randomly subdivided into two groups of 4 teeth each according to the carrier-based obturation system used.

Group 1: GuttaCore, a metal Verifier (Dentsply Tulsa) corresponding to the size of the final file used (#30/0.04) was placed 0.5mm short of the working length and checked with a periapical radiograph. A thin layer of Tubli-Seal root canal sealer (SybronEndo, Orange, CA, US) was used to coat the root canal walls. The obturator was placed into ThermoPrep[®] Plus obturator oven to soften the two obturators used and then inserted to the pre-determined working length with slight pressure. The handle of the obturators with the remnants of the obturation material around was removed with a round diamond bur.

Group 2: Experimental HA/PE obturation system. The root canals were obturated in a similar manner to the samples in the Group 1; the main difference is that the temperature used to soften the experimental obturator was reduced to 100°C by the incorporation of a thermo-regulator connected to the current ThermoFil[®] oven to monitor temperature control. Afterwards, the obturated roots were stored at 37°C with 100% humidity to allow complete setting of the sealer.

Micro-CT imaging

The teeth were scanned using a GE Locus SP μ CT scanner (General Electric, London Ontario, Canada) with an X-ray tube voltage of 80kVp and a current of 80uA. A 0.1 mm aluminium filter was used to attenuate the X-ray source. The specimens were immobilised using cotton gauze and scanned to produce $6.5 \times 6.5 \times 6.5 \mu\text{m}$ voxel size volumes. A full 360 degree scan provided 900 views (i.e. one view every 0.4 degrees) with an exposure of 3 seconds (with four averages per view). All the scans were imported by Scan IP (Simple ware®, Exeter, UK) as a stack of images where a segmentation had been carried on each individual slice according to the pixel density. The samples were further characterised by making three-dimensional reconstructions.

4.2.2.2 Measurement of the thickness of gutta-percha coating of the experimental HA/PE and commercial obturators

Five samples of GuttaCore (Dentsply Tulsa) and HA/PE (experimental) obturators were prepared from the middle third of the obturators as shown in Figure 4.2. The obturators were scanned using a GE Locus SP μ CT scanner (General Electric) using the same settings described above. Two masks were produced to represent the gutta-percha coating and the carriers. The thickness of GP around its carrier was calculated for nine slices of each sample. Four identical fixed points (“12, 6, 3 and 9”) on each slice were identified for thickness measuring in both samples in order to make a comparison between gutta-percha coated (GC) and (HAPE) cores as shown in Figure 5.15 C, D, E, and F. The results of the μ CT study were statistically analysed using ANOVA test followed by Bonferroni test. The mean difference was significant at the 0.05 level.

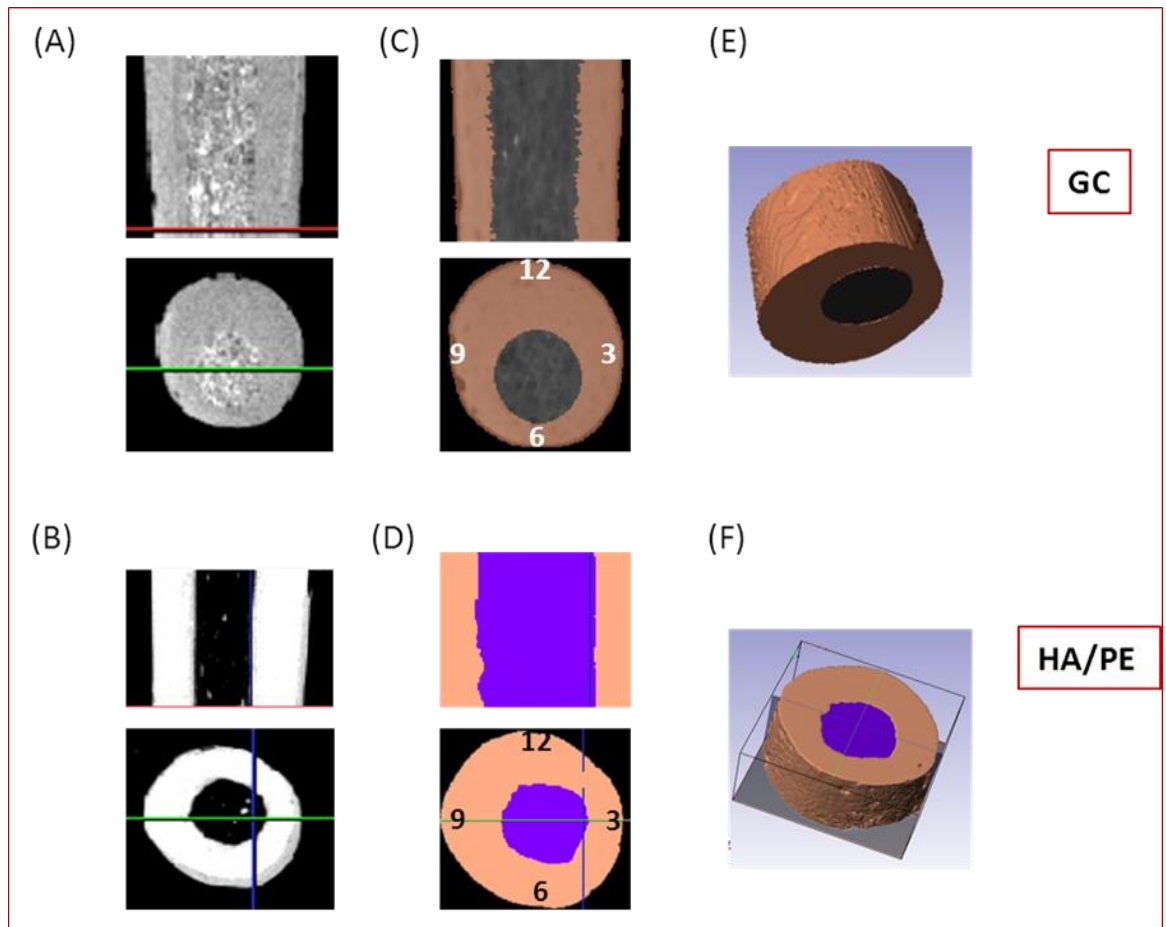


Figure 4.2: Micro-Computed Tomography images prepared from the middle third of the GC and HA/PE obturators. (A+B) μ CT scans of the GC and HA/PE systems, respectively showing slice selection from the middle third. (C+D) μ CT slices of GC and HA/PE respectively segmented by Scan IP[®] using different masks. (E+F) 3D reconstruction of GC and HAPE systems using Scan IP[®] based on voxel density.

4.2.3 Bondability of gutta-percha coating around the experimental and commercial carriers

Three types of endodontic obturators were tested: Thermafil® (Dentsply Tulsa), GuttaCore (Dentsply Tulsa), and the experimental hydroxyapatite polyethylene obturator (HA/PE) as shown in Table 4.1

Table 4.1: Endodontic obturators used in the study

Product	Manufacturer	Material
Guttacore 30/0.04	Tulsa	Cross-linked gutta-percha
Thermafil® 30/0.04	Tulsa	Polyethylene terephthalate (Vectra)
HA/PE 30/0.02	Experimental	Hydroxyapatite-polyethylene-strontium oxide

Ten carrier sizes 30/0.04 with a length of 25mm from each carrier type were selected. The portion of each carrier coated with gutta-percha was divided into three parts, 5mm long (coronal, middle and apical). One slice (1mm thick) was obtained from the junction point of the coronal and middle portion of each carrier using a low speed diamond wafering blade (Isomet; Buehler). The diameters of the carriers pre-measured using a digital calliper (Maplin Electronics, Rotherham, UK) were found to be $0.66\text{mm} \pm 0.04\text{ mm}$ in the case of GuttaCore and Thermafil® obturators and $0.66 \pm 0.02\text{ mm}$ in the case of the experimental HA/PE carriers. Each slice was fixed to the aperture of a plastic syringe (Terumo®, Leuven, Belgium) 1.5 mm in diameter in a vertical position using a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, USA). A 0.5 mm-round orthodontic wire was attached to the plunger of an Instron machine (Instron model 5569 A, High Wycombe, UK) in order to load the carrier at a constant crosshead speed of 0.5mm/min. A silicon-based material (Metrodent, Huddersfield, UK) was used to fix the syringe with its long axis parallel to the long axis of the plunger. On the loading machine, each slice was positioned with the

larger side of the carrier segment placed facing the punch tip as shown in Figure 4.3. Bond failure was considered. The displacement of the carrier segment from the gutta-percha was classified as a bond failure. Push-out strength data were determined in MPa by dividing the load in Newtons by the bonded surface area (SL) in mm². SL was calculated using the formula (Giachetti *et al.* 2012): $SL = \pi (R1 + R2) \sqrt{(R1 + R2)^2 + h^2}$

Where $\pi = 3.14$, (R1) is the apical carrier radius (base), (R2) is the coronal carrier radius (top), and h is the height of the slice.

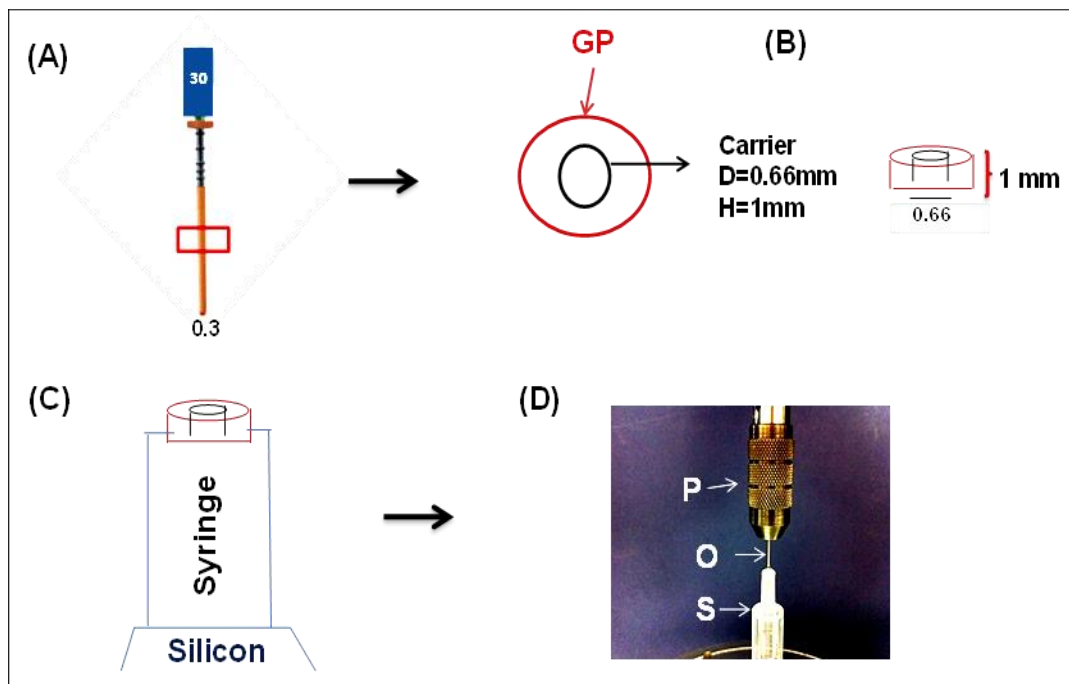


Figure 4.3: Schematic representation of the push-out test procedure. (A) The middle part of the endodontic obturator is chosen for the test. (B) Slices (height 1mm, diameter 0.66mm) are obtained. (C) The slice is fitted into the aperture of a customized syringe. (D) The plunger, of the Instron machine is equipped with an orthodontic wire (0.5 mm in diameter), and aligned with the slice to be tested.

4.3 Results

The composite fibres were fabricated using the composition shown in Table 2.2 (Chapter 2, Section 2.2.2.1) then successfully coated with gutta-percha to form the carrier-based root canal obturator as shown in Figure 4.4

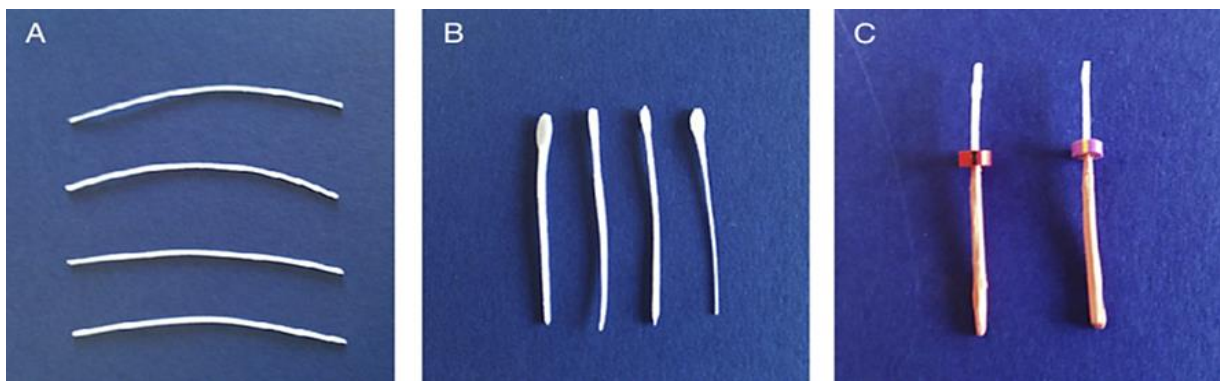


Figure 4.4: A) Polyethylene-hydroxyapatite composite fibres in different diameters, B) tapers and C) obturators

Modified Thermafil[®] oven

A specifically designed heater was constructed in this study and used to soften the experimental HA/PE endodontic obturator and the commercial obturators as well, as shown in Figure 4.5. The specifically-designed carrier-based oven is a modified Thermaprep[®] Plus Oven (Dentsply, Maillefer). A thermo-regulator was incorporated into the design of the Thermafil oven to monitor the temperature which was required for softening the gutta-percha of the various carrier types. The thermo-regulator could be disconnected from oven effortlessly in order to allow the conventional use of the Thermafil oven.

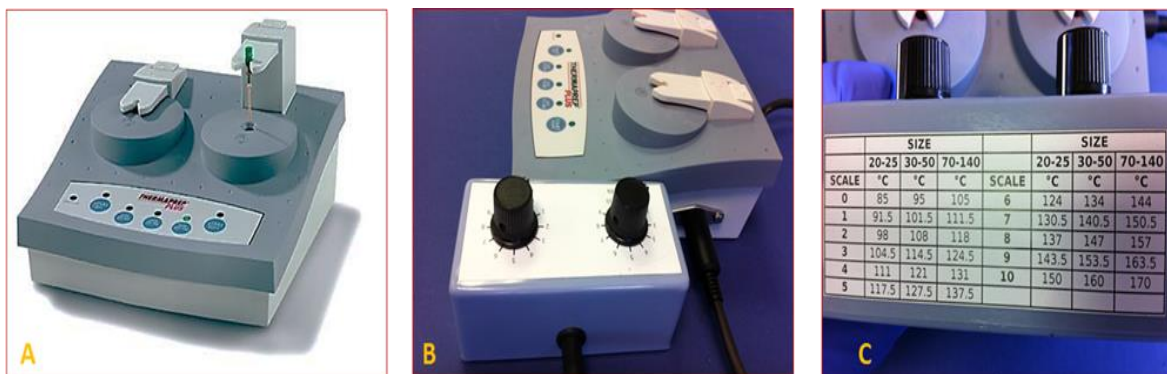


Figure 4.5: A) Conventional Thermafil[®] oven, B) A modified Thermafil oven which has been attached to a thermoregulator to control the temperature of the obturator. C) Temperature scales for the different obturator sizes

4.3.1 FTIR spectral analysis

The silanation of the hydroxyapatite was confirmed by the FTIR spectra of the silanated and non silanated HA as shown in Figure 4.6. The FTIR spectral wave range was 4000-650 cm^{-1} . The silanated HA exhibited a characteristic peak at 1720 cm^{-1} attributed to the carbonyl group arising from the gamma methacryloxypropyltrimethoxysilane coupled to the hydroxyapatite, with other peaks at 1637 cm^{-1} , 1460 cm^{-1} and 1300 cm^{-1} due to the $-\text{C}=\text{C}-$, $\text{C}-\text{C}-$ and $\text{Si}-\text{O}$ stretching frequencies respectively. Thus, the presence of SiO & $\text{C}=\text{O}$ absorption bands confirmed the availability of the silanation procedure on the surface of hydroxyapatite. The FTIR spectra of both LDPEHASR20 and LDPEHASR40 are shown in Figure 4.7, they were quite similar to each other as expected, however, the intensity of the phosphate peaks varied due to the compositional changes. The two sharp peaks at 2848 cm^{-1} and 2916 cm^{-1} were due to $-\text{CH}_2$ stretching and $-\text{CH}_2$ deformation arising from the polyethylene segments, respectively with absorption bands at 962, 1022 and 1091 cm^{-1} attributed to the phosphate groups, ($\text{P}-\text{O}$, stretching) in the hydroxyapatite.

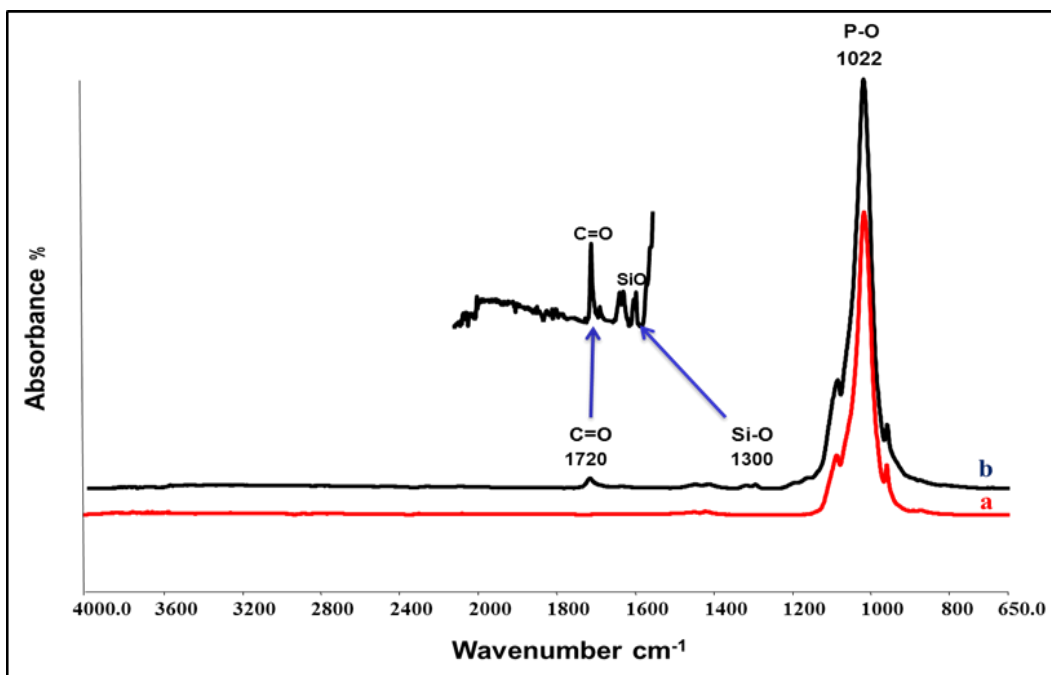


Figure 4.6: FTIR spectra of the hydroxyapatite particles (a) before silane treatment (red line), (b) after silane treatment (black line), inset showing the characteristic peaks of the silane coupling agent on the surface of HA.

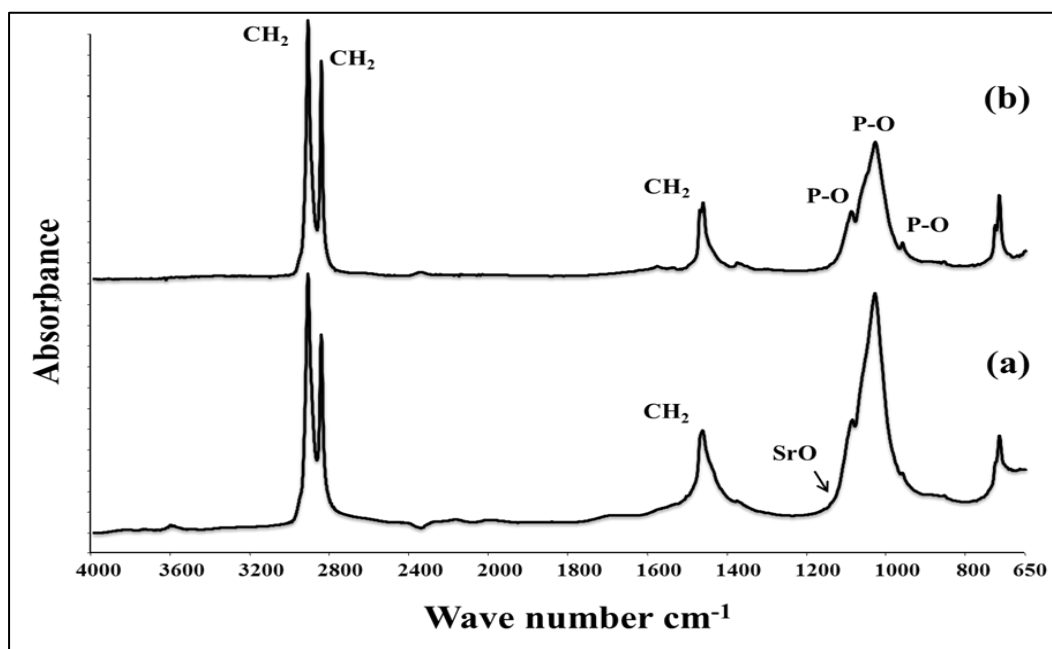


Figure 4.7: FTIR spectra of a) LDPEHASRO 40 and b) LDPEHASRO 20.

4.3.2 Tensile test (tensile strength and elastic modulus)

The tensile strength of the LDPE containing 20% HA and 40% HA composites were 10.89 ± 0.28 MPa and 10.80 ± 1.39 MPa, respectively, with no significant difference between HA/PE groups ($P > 0.05$); however, the plastic carrier of Therafil[®] showed a strength of 36.9 ± 0.98 MPa which was significantly higher than that of LDPEHASR20 and LDPEHASR40 composite carriers ($P < 0.001$). The elastic modulus of the Therafil[®] carrier (10.4 ± 3.33 GPa,) was significantly higher ($P < 0.001$) than that of PE containing 20%HA 0.61 ± 0.11 GPa and 40%HA composites 0.91 ± 0.09 GPa. The results are summarized in Table 4.2 and shown in Figure 4.8 & 4.9. Statistical analysis of the tensile strength and elastic modulus data was performed using GraphPad Prism software version 5 (GraphPad Software Inc, La Jolla, CA). One-way analysis of variance followed by Tukey multiple comparison post hoc tests were used to analyse the data. The level of significance was set at $P = 0.05$.

Table 4.2: Mean tensile strength and elastic modulus of the experimental composites and Therafil[®] plastic carrier (control) with standard deviations shown in parentheses, (n=6)

Material	Tensile strength	Elastic modulus (GPa)
LDPEHASRO 20	$10.89 \pm (0.82)$	$0.61 \pm (0.11)$
LDPEHASRO 40	$10.80 \pm (1.39)$	$0.91 \pm (0.09)$
Therafil [®]	$36.39 \pm (0.98)$	$10.4 \pm (3.33)$

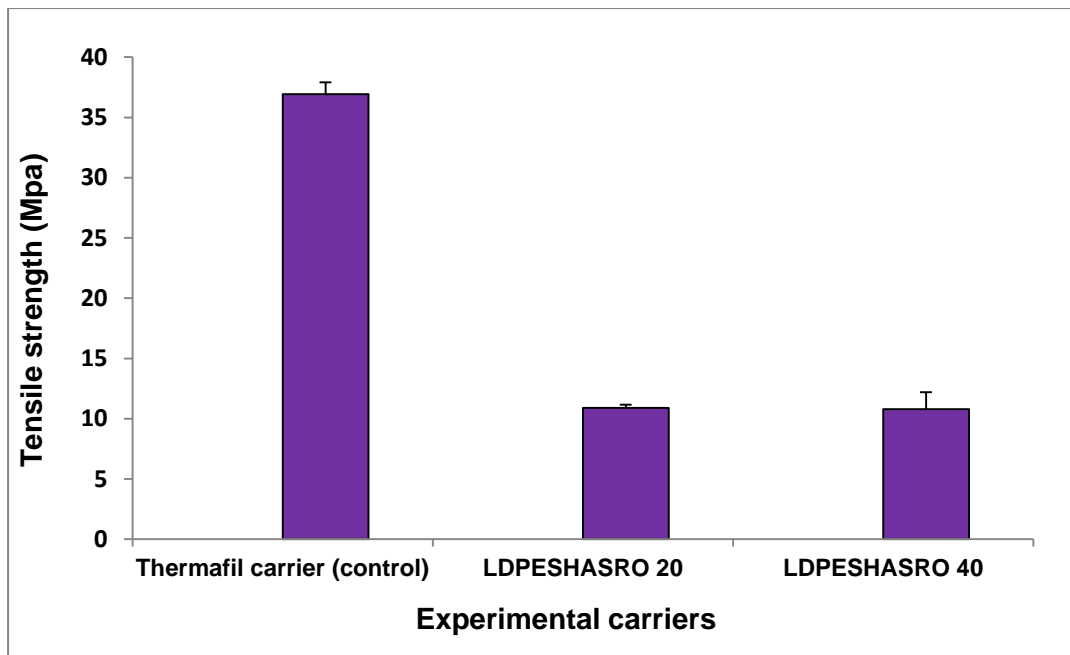


Figure 4.8: Tensile strength of experimental carriers and Thermafil[®] carrier (control).

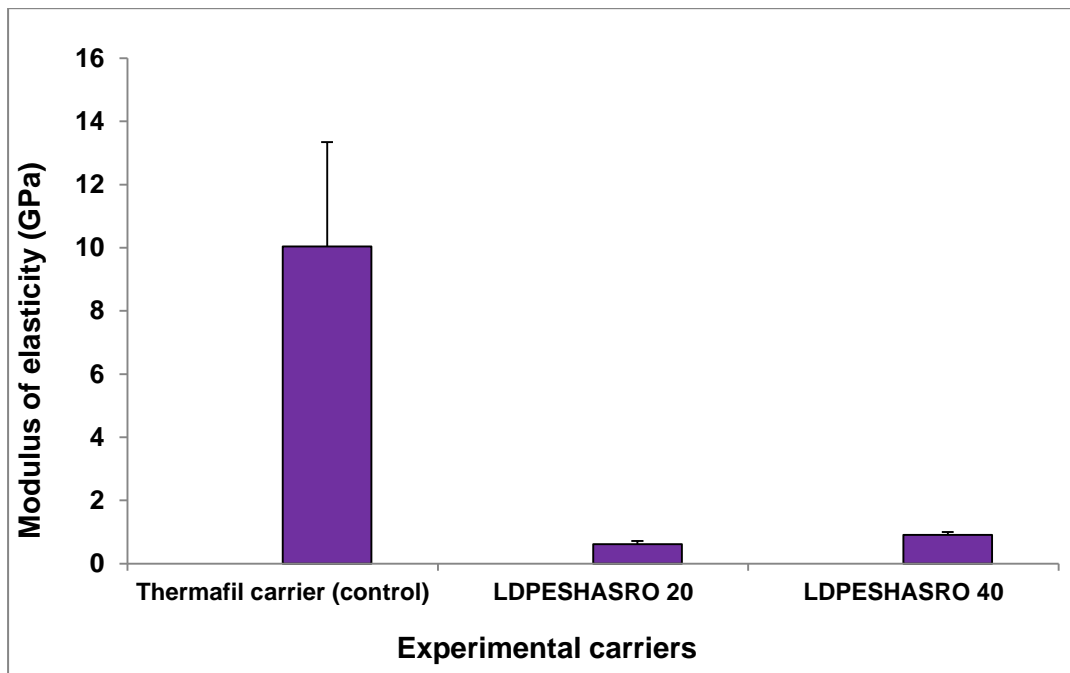


Figure 4.9: Elastic modulus of experimental carriers and Thermafil[®] carrier (control).

4.3.3 Thermal properties: Differential Scanning Calorimetry (DSC) & Dynamic Mechanical Analysis

4.3.3.1 DSC analysis

The DSC measurements showed that the LDPEHASR20 and LDPEHASR40 composites had a melting temperature (T_m) range between 111 and 115°C in contrast to the 350°C exhibited by the plastic carrier used for Thermanfil®. These findings are illustrated in Table 4.3 & Figure 4.10. The lower T_m indicated that the temperatures required to remove the experimental carriers were more suited to the clinical situation.

Table 4.3: Mean melting temperatures of the polyethylene-hydroxyapatite composites and Thermanfil® plastic carrier as core carrier for GP with standard deviations shown in parentheses (n=3).

material	$T_m/^\circ\text{C}$
LDPEHASRO 20	115 \pm (2)
LDPEHASRO 40	111 \pm (1)
Thermanfil® plastic carrier	350 \pm (1)

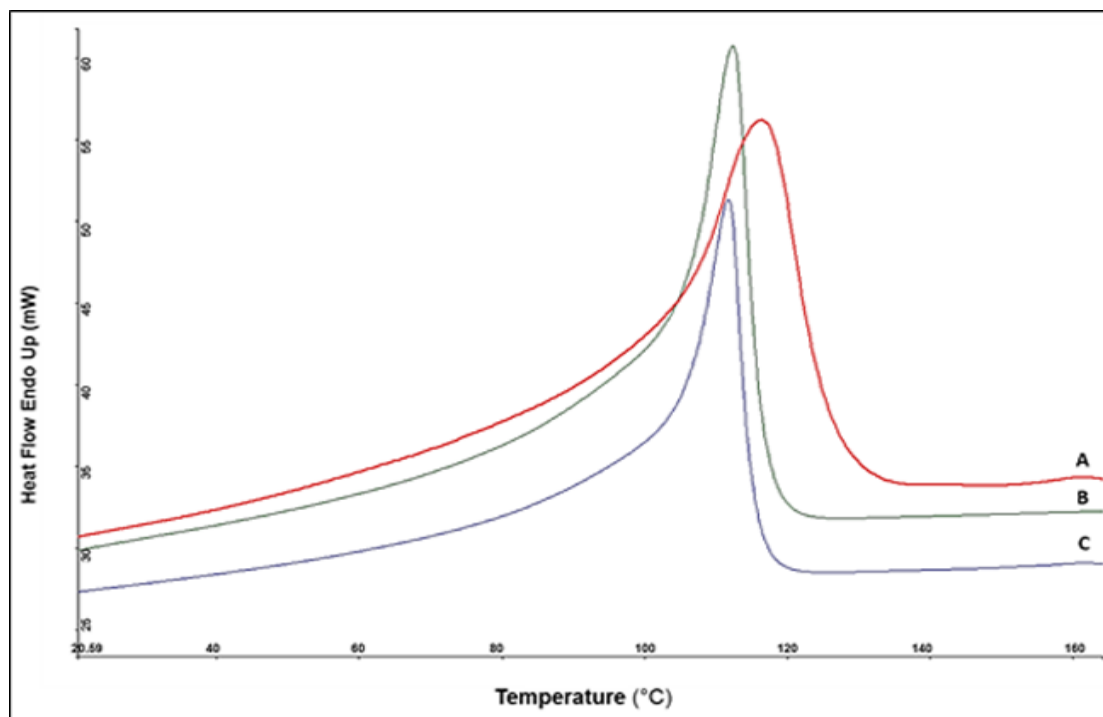


Figure 4.10: A typical DSC thermogram of the second heating cycles of A) LDPEHASRO 20, B) LDPE and C) LDPEHASRO 40, showing the melting points.

4.3.3.2 DMA

Figure 4.11 shows thermograms for low density polyethylene (LDPE), experimental composites of LDPE-HA-SrO (20% and 40% by weight of filler) illustrating the storage and loss moduli. Table 4.4 represents the findings of the dynamic storage modulus and loss modulus of experimental composites versus the temperature. The storage modulus of the composites was almost stable or slightly decreased at temperatures below the glass transition and then a dramatic drop was observed at glass transition temperature point as expected. Universally, the storage modulus of LDPEHASRO 40 was significantly higher than those of pure LDPE and LDPEHASRO 20. Increase in the amount of the filler in the composite material lead to an increase in the dynamic elastic modulus.

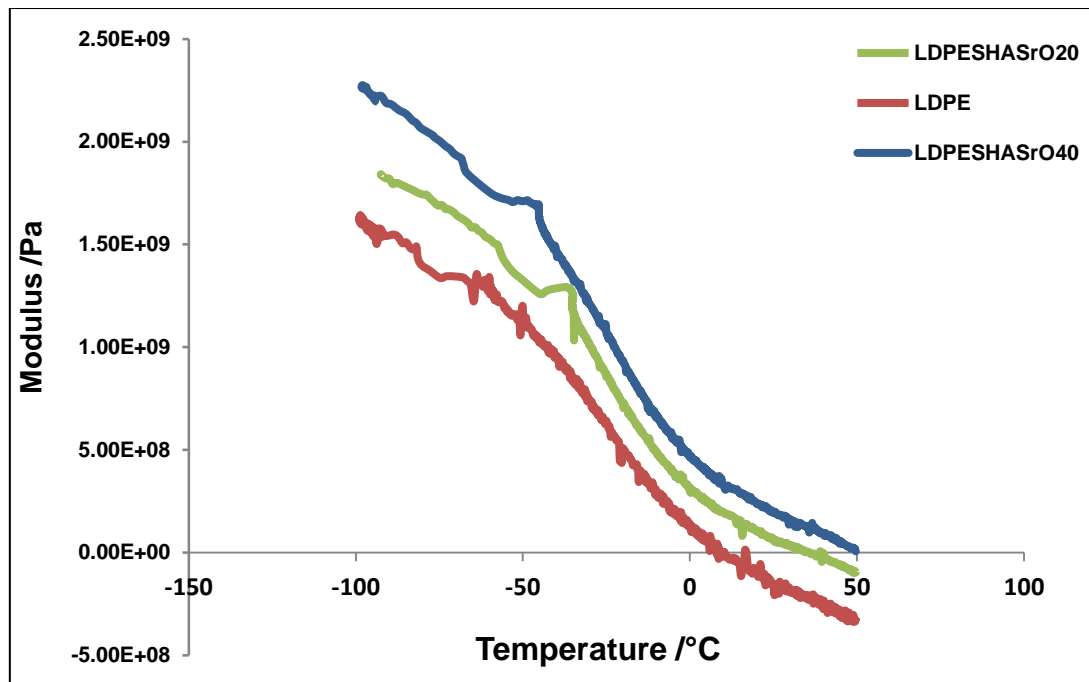


Figure 4.11: DMA thermograms for LDPE, experimental composites of LDPE-HA-SrO (20 and 40% by weight of filler) showing the storage and loss moduli.

Table 4.4: Storage, loss moduli and glass transition temperatures of the experimental composites as core carrier for GP

Material	Tg/°C	Storage modulus/Pa (-92°C)	Storage modulus/Pa (25°C)	Loss modulus/Pa (-92°C)	Loss modulus/Pa (25°C)
LDPE	-66.7	1.517×10^9	-1.51×10^8	3.1×10^7	-3.24×10^7
LDPESHASRO 20	-61.3	1.83×10^9	6.88×10^7	4.38×10^7	2.28×10^7
LDPESHASRO 40	-67.8	2.222×10^9	1.94×10^8	6.39×10^7	3.69×10^7

4.3.4 X-ray contrast and microscopy

The radiographs shown in Figure 4.12 A-C demonstrate that the specifically-designed core carrier was sufficiently radiopaque and could be easily discriminated from gutta-percha, particularly when examined after repositioning of the root filled tooth into a dry mandible. Furthermore, the images obtained from light microscopy of the fibres showed a homogenous dispersion of the HA filler within the matrix and a slight irregularity of the external surfaces indicating that the fibres can be coated with GP obtaining good micro-mechanical adhesion as shown in Figure 4.12 D

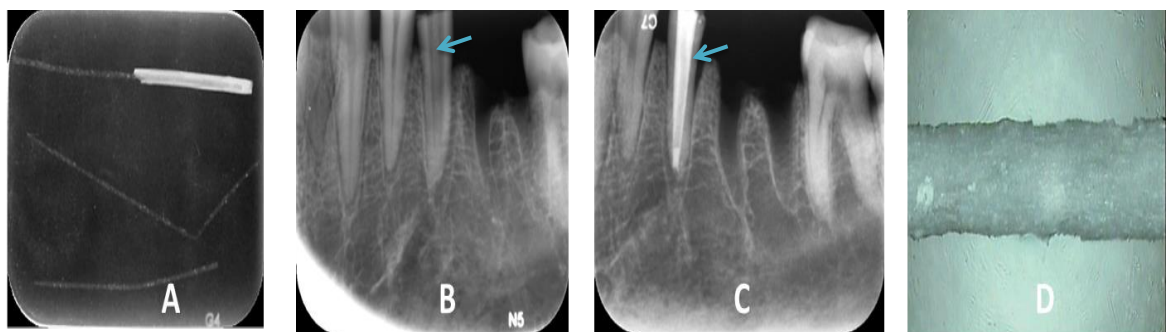


Figure 4.12: Radiograph showing the newly designed carrier made of LDPEHASR20 (A), the carrier LDPEHASR20 inserted in the root canal space of an extracted tooth positioned into an alveolus of a dry mandible (*arrow*) (B), LDPEHASR20 carrier coated with gutta-percha inserted in the root canal space of an extracted tooth positioned into an alveolus of a dry mandible (*arrow*) (C), image of the fabricated LDPEHASR20 composite before gutta-percha coating (D), (2 X).

4.3.5 Fluid filtration

The mean and standard deviation of fluid flows for the tested materials (GC and HA/PE) applied in simulated root canals at 30, 60, 90 min are shown in Figure 4.13, 4.14 and Table 4.5, respectively. There was no leakage in the negative control, while all the groups with/without sealer showed leakage of the fluid over time. In simulated root canals, the GuttaCore exhibited a superior sealing ability to the HA/PE at 30 min of evaluation ($p < 0.05$) both when applied with and without

the use of the sealing agent; no significant change was observed between 60 and 90 min ($p>0.05$). However, neither obturator system applied with nor without the use sealer displayed a significant difference ($p>0.05$) in the ultimate leakage evolution (90min) as shown in Table 16. The data (μLmin^{-1}) were statistically analysed one-way ANOVA and Dunn's multiple comparison test ($\alpha=0.05$).

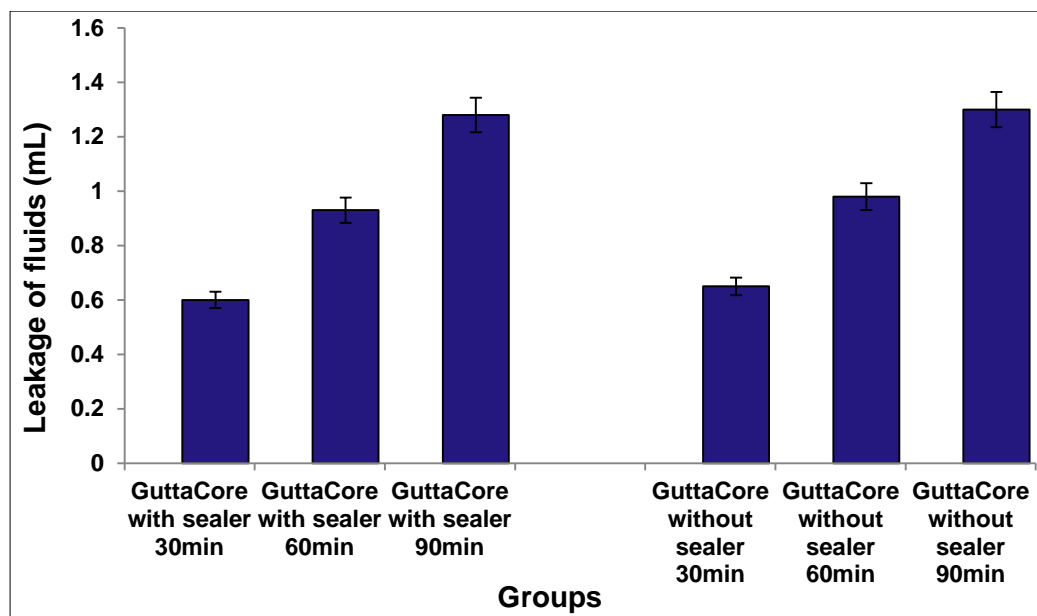


Figure 4.13: Fluid filtration of GC obturation with/without sealer in simulated canal in (mL/30, 60, 90 min).

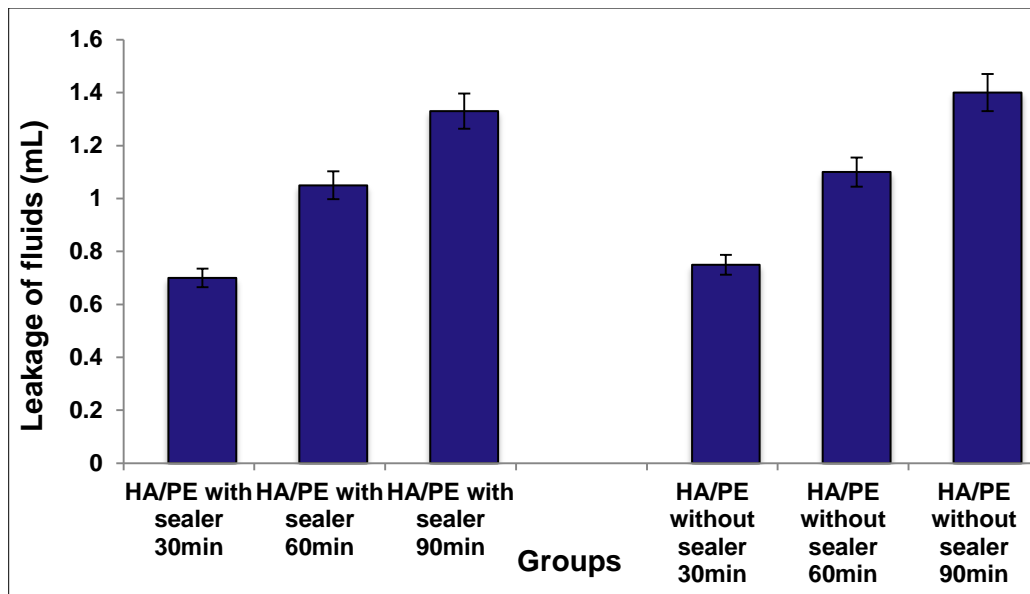


Figure 4.14: Fluid filtration of HA/PE obturation with/without sealer in simulated canal in (mL/30, 60, 90 min).

Table 4.5: Mean fluid filtration in simulated root canal during three time measurements determined at 30, 60, 90 minutes with standard deviations shown in parentheses (n=6)

Groups	Leakage (mL*30min ⁻¹)	Leakage (mL60min ⁻¹)	Leakage (mL90min ⁻¹)
GuttaCore (Tulsa, OK, USA) with sealer	0.6 ± (0.06)	0.93 ± (0.08)	1.28 ± (0.07)
GuttaCore (Tulsa, OK, USA) without sealer	0.65 ± (0.05)	0.98 ± (0.07)	1.3 ± (0.06)
HA/PE without sealer	0.75 ± (0.05)	1.1 ± (0.08)	1.4 ± (0.06)
HA/PE with sealer	0.7 ± (0.06)	1.05 ± (0.08)	1.33 ± (0.08)
Negative control	0*	0	0
Positive control	maximum*	maximum	maximum

ml* represents the displacement of air bubble in the capillary tube of distilled water

0* refers to no leakage, Maximum* refers to gross leakage

While in human root canals, both GuttaCore and the HA/PE exhibited significantly higher sealing ability both at 30 min of evaluation and at ultimate leakage ($p<0.05$) when applied with the use of the sealing agent; no significant difference was observed between the two materials used without sealers. The results are illustrated in Table 4.6, Figures 4.15 & 4.16

Table 4.6: Mean Fluid filtration of two carrier based root canal obturation in single rooted teeth in (mLmin^{-1}) during three time measurements (30, 60, 90 minutes) with/without root canal sealer, with standard deviations shown in parentheses ($n=6$).

Groups	Leakage ($\text{mL} \cdot 30\text{min}^{-1}$)	Leakage ($\text{mL} \cdot 60\text{min}^{-1}$)	Leakage ($\text{mL} \cdot 90\text{min}^{-1}$)
GuttaCore (Tulsa, OK, USA) without sealer	$0.71 \pm (0.04)$	$1 \pm (0.06)$	$1.33 \pm (0.05)$
GuttaCore (Tulsa, OK, USA) with sealer	$0.56 \pm (0.08)$	$0.95 \pm (0.05)$	$1.28 \pm (0.07)$
HA/PE without sealer	$0.75 \pm (0.05)$	$1.05 \pm (0.05)$	$1.36 \pm (0.05)$
HA/PE with sealer	$0.55 \pm (0.5)$	$0.88 \pm (0.07)$	$1.21 \pm (0.09)$
Negative control	0.00*	0.00	0.00
Positive control	maximum*	maximum	maximum

ml* represents the displacement of air bubble in the capillary tube of distilled water

0* refers to no leakage, Maximum* refers to gross leakage

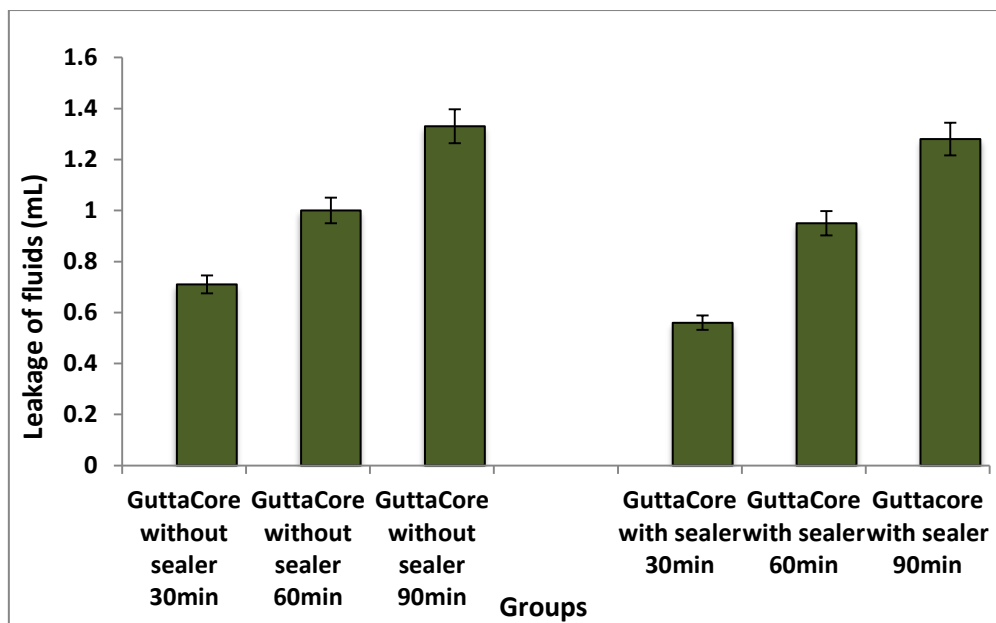


Figure 4.15: Fluid filtration of GC obturation with/without sealer in extracted teeth in (mL/30, 60, 90 min).

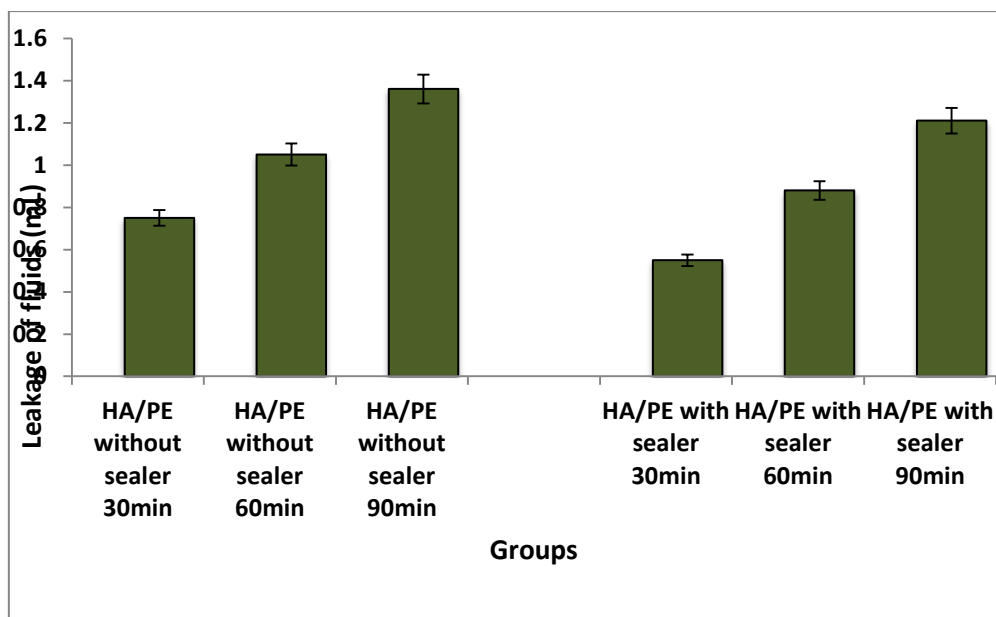


Figure 4.16: Fluid filtration of Ha/PE obturation with/without sealer in extracted teeth in (mL/30, 60, 90 min).

In summary, simulated canals obturated with polyethylene hydroxyapatite obturators showed more fluid filtration than GC ($p < 0.05$) during the first 30min and 60 min with and without sealer. No significant differences ($p > 0.05$) were observed between the two materials when applied in human root canals at different intervals, however, the root canal sealer tended to minimise the fluid flow.

4.3.6 Voids volume qualification of the experimental and commercial obturators

The findings and images obtained for GC and the experimental PE/HA, are shown in Table 4.7 & Figures 4.17 & 4.18. The percentage of the volume of voids was obtained by dividing the total volume of voids by the total volume of root canal obturation, calculated by the μ CT (IP) software. GC obturation showed a lower percentage of voids volume (1.54%) than the experimental HA/PE obturation (2.3%). Overall, canals obturated with GC presented the lowest percentage of voids, whereas canals obturated with PE/HA showed a higher percentage of voids. PE/HA root obturation showed more voids than GC, particularly in the apical region. The data obtained from μ CT were analysed with ANOVA followed by Bonferroni test. The void volume percentage in the GuttaCore system was non-significantly lower ($P > 0.05$) than that of experimental PE/HA system.

Table 4.7: the percentage of voids volume in the root canal space for experimental PE/HA and GuttaCore obturation systems

Product	Total volume of voids (mm ³)	Total volume of root obturation (mm ³)	Overall voids (%)
GuttaCore	0.319	20.04	1.54%
LDPE/HA	0.848	34.70	2.3%

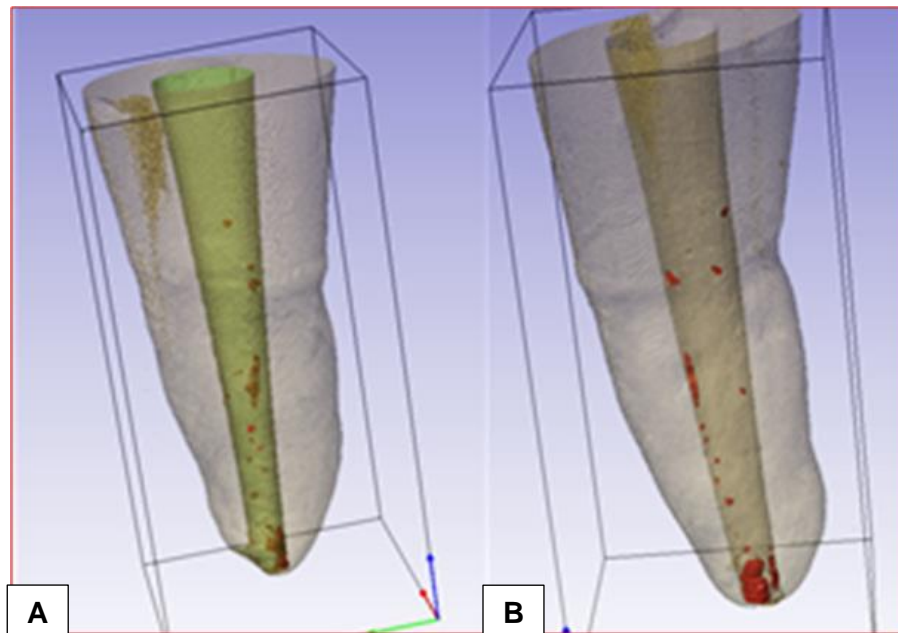


Figure 4.17: Three-Dimension models of Micro-CT images showing the voids in the root canal obturation (red) for GuttaCore (A) and experimental PE/HA obturators (B).

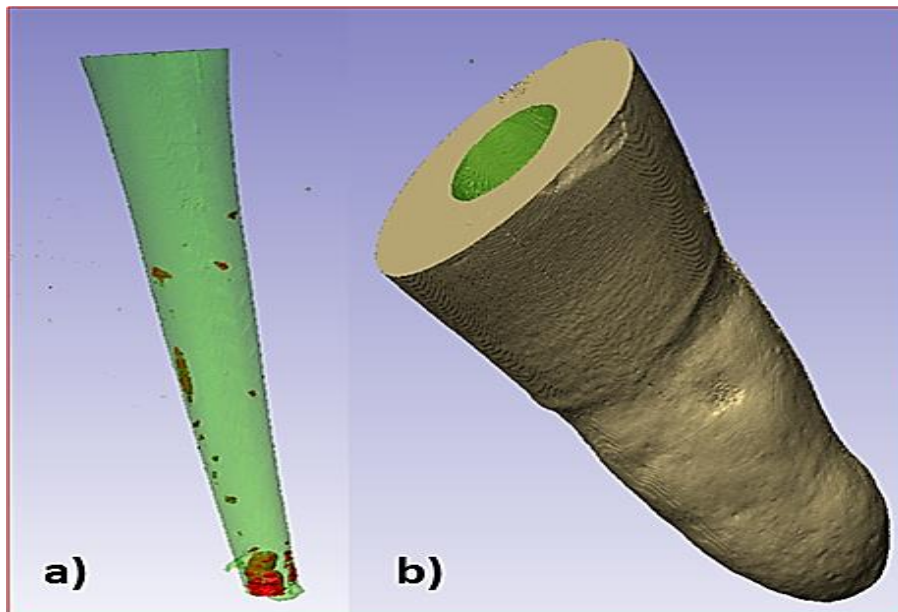


Figure 4.18: Three-Dimension models of Micro-CT images showing the PE/HA obturator (green) with voids (red) in (a), and the single rooted tooth prepared for PE/HA obturator.

4.3.7 Measurement of the thickness of gutta-percha coating of the experimental and commercial obturators

The results of the μ CT scans demonstrated that the thickness of gutta-percha coating on the polyethylene hydroxyapatite (PE/HA) system was more uniform and homogenous than that of GuttaCore system, suggesting void creation which were lower in the PE/HA system in comparison to GuttaCore system. The thicknesses of gutta-percha around the GuttaCore system were found to be 0.591 ± 0.034 , 0.164 ± 0.023 , 0.376 ± 0.027 and 0.357 ± 0.033 mm, respectively. However, the PE/HA system showed thicknesses of gutta-percha from “12, 6, 3, 9” watch-hour positions at 0.535 ± 0.04 , 0.607 ± 0.042 , 0.419 ± 0.03 and 0.584 ± 0.039 mm, respectively. The difference in thickness of gutta-percha between GC system and PE/HA was significant ($P < 0.05$), particularly in point “6” and point “9”. These findings are illustrated in Table 4.8 & Table 4.9.

Table 4.8: Thicknesses of gutta-percha around the carrier for the GC obturation system (4 point measurements; 12, 3, 6, 9), (in mm).

1 st point (12) mm	2 nd point (6) mm	3 rd point (3) mm	4 th point (9) mm
0.617	0.161	0.361	0.349
0.554	0.162	0.38	0.324
0.604	0.156	0.361	0.331
0.604	0.143	0.38	0.318
0.629	0.15	0.343	0.368
0.555	0.206	0.43	0.337
0.629	0.156	0.386	0.411
0.592	0.162	0.349	0.399
0.539	0.206	0.399	0.38
M 0.591	0.164	0.376	0.357

Table 4.9: Thicknesses of gutta-percha around the carrier for the HA/PE obturation system (4 point measurements; 12, 3, 6, 9), (in mm).

1st point (12) mm*	2nd point (6) mm	3rd point (3) mm	4th point (9) mm
0.602	0.547	0.377	0.651
0.59	0.584	0.395	0.584
0.517	0.59	0.42	0.614
0.541	0.572	0.421	0.596
0.56	0.608	0.42	0.596
0.511	0.59	0.414	0.547
0.511	0.639	0.401	0.511
0.493	0.663	0.445	0.584
0.493	0.673	0.481	0.578
M 0.535	0.607	0.419	0.584

*mm** represents millimetre

4.3.8 Bondability of gutta-percha coating to the carrier materials (push-out bond strength)

The push-out results of the test materials showed that the mean push-out bond strength of the experimental obturator, GuttaCore and Thermafil[®] obturators was 6.84 ± 0.96 , 3.75 ± 0.75 and 1.5 ± 0.63 MPa, respectively. The push-out strength of the experimental carriers was found to be significantly higher ($P < 0.001$) than those of GuttaCore and Thermafil[®] whilst GuttaCore push-out bond strength was significantly higher than Thermafil[®] ($P < 0.001$) as shown in Figure 4.19.

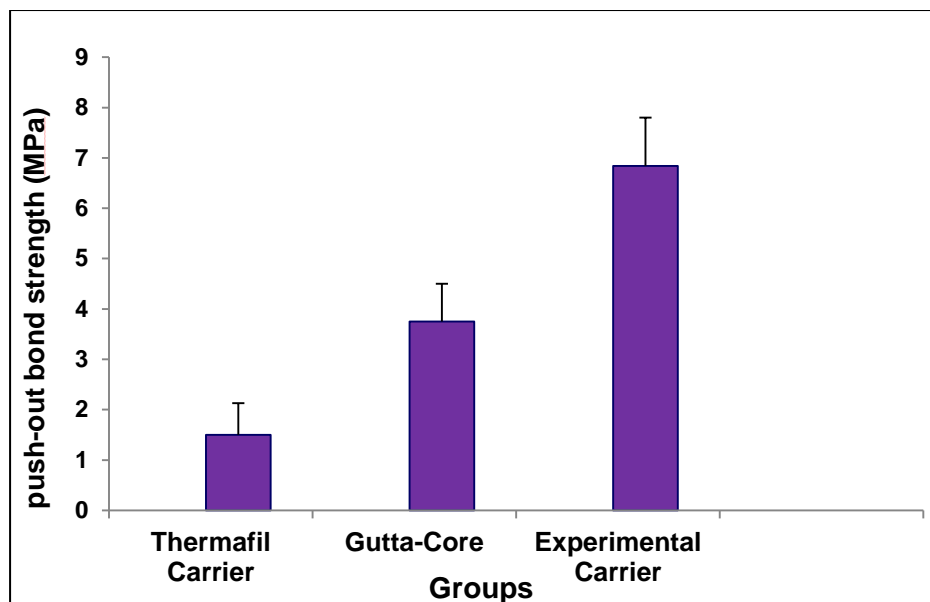


Figure 4.19: Push-out bond strength of the experimental obturators compared with Thermafil and GuttaCore (MPa).

4.3.9 Technical procedure required for removal of polyethylene-hydroxyapatite core for carrier-based root canal obturation

The technical approach for carrier removal is based on heat application followed by immediate insertion of a hedström file size #20 or #25 to remove both GP and carrier materials from the root canal space. Heat was applied to the experimental obturator (carrier with GP) using a hot endodontic instrument to soften the coronal part of the carrier at a temperature of 85-90°C avoiding the separation of the apical part. Afterwards, an H-file was introduced to the root canal to retrieve the heated plastic carrier with a pulling action as shown in Figure 4.20.

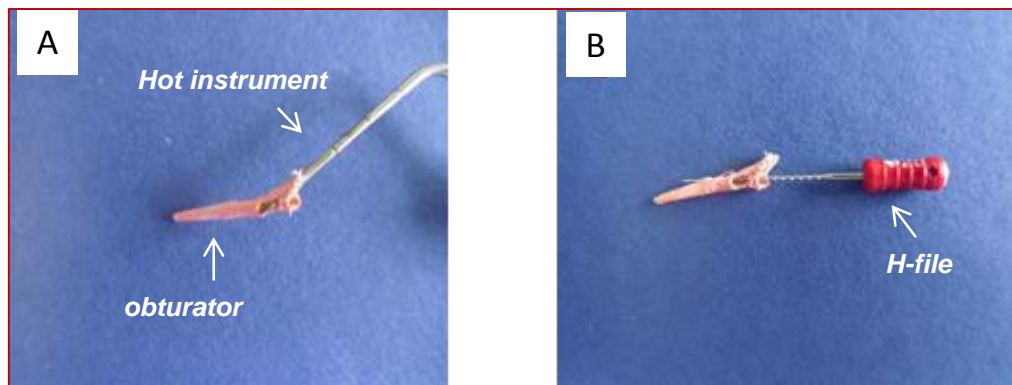


Figure 4.20: A hot instrument was used to soften the coronal part of the obturator (a), followed by immediate insertion of a hedström #25 file and pulling.

4.4 Discussion

Carrier-based root canal obturation is one of the most common techniques used for root canal obturation enhancing, adaptability of warm gutta-percha to the root canal wall and into lateral canals in a controlled and fast manner (Li *et al.* 2014a). However, problems reported by clinicians using carrier-based systems include the difficulties in removing the carrier in case of retreatment or post space preparation (Kytridou *et al.* 1999) and lack of apical control (Gutmann *et al.* 1993). Thus, specifically designed composite fibres made of LDPE, HA and SrO were developed for use as carriers using a melt extrusion process. A single screw extruder was employed for processing the experimental composite fibres. The fibres were drawn and tapered as required using appropriate dies. The extruded fibres were then successfully coated with a thin layer of gutta-percha using chloroform, which was used to dissolve the gutta-percha. Owing to their thermoplasticity, processability, and high flexibility, polyethylene was used in this study in conjunction with hydroxyapatite (HA) fillers. Two concentrations of 20% and 40% hydroxyapatite particles were incorporated into LDPE in an effort to improve and optimise the mechanical properties of the composite material and to impart radiopacity; hence low and high concentrations of HA were used although

the maximum percentage of filler was limited to 40%. It is reported that the quantity of the ceramic filler is significant parameter which assists in the ability to tailor the flexibility of the carriers. The hydroxyapatite particles were treated with a silane coupling agent to enhance the interfacial bonding between filler and matrix, thus enhancing the overall properties of the composite (Deb *et al.* 1996). Hydroxyapatite was treated with a coupling agent, gamma methacroyloxy trimethoxy silane to render it more hydrophobic thereby enhancing the interfacial adhesion between the filler and the polyethylene, which is inherently hydrophobic. The silanol groups condense with the hydroxyl groups on adsorption on the surface of the hydroxyapatite particles and the subsequent thermal treatment leads to formation of siloxane bridges, leading to a hydrophobic surface and thereby facilitating the blending of the inorganic and organic matrices in order to obtain a better adhesion between the two components.

FTIR spectral analyses confirmed the presence of HA and PE in the composites with no evidence of degradation occurring throughout the processing. Two sharp peaks at 2848 cm^{-1} and 2916 cm^{-1} were attributed to $-\text{CH}_2$ stretching and $-\text{CH}_2$ deformation of polyethylene, respectively were observed in the FTIR spectra and absorption bands at 962 cm^{-1} and 1022 cm^{-1} , 1091 cm^{-1} , which are accounted for the phosphate groups of the hydroxyapatite were also characterised, indicating the presence of polyethylene matrix and hydroxyapatite filler within the tested composite, respectively. Similar findings have been reported in earlier studies, wherein the characterisation peaks of LDPE were reported (Santhoskumar *et al.* 2010). The presence of the coupling agent on the surface of hydroxyapatite particles was also confirmed by the appearance of the peak at 1720 cm^{-1} , which was attributed to the carbonyl group and the peak in the region of $1300\text{--}1200$

cm^{-1} , assigned to silicon oxide group. The characterisation peaks of silanated hydroxyapatite reported by FTIR in this current work were supported by an early study (Deb *et al.* 1996).

The melting temperatures of the hydroxyapatite reinforced low density polyethylene composites were characterised using DSC. The melting point of the newly developed composites was determined, around 111°C and the addition of HA filler showed no significant changes on the melting temperature of PE. These DSC results are in accordance with the observations of a previous study (Madera-Santana *et al.* 2010), wherein the thermal properties of LDPE-agar bio-composites were characterised and showed no changes on the T_m of PE by inclusion of agar. The clinical relevance here is that it was possible to remove the experimental core carrier easily and safely from the root canal space using a heat source at a temperature of 85°C to 100°C in cases of retreatment. Such temperatures are far below the threshold of 200°C that is associated with the risk of damage to the periodontal tissues (Lipski & Woźniak 2003). This is not possible with the current Thermafil[®] plastic carriers, which have a melting point, around 350°C. The retrievability of Thermafil[®] carriers is relatively difficult, particularly plastic carriers size #40 or smaller because they are not soluble in commonly used solvents such as chloroform (Parker & Glickman 1993) because they are made of Vectra, a liquid crystalline polymer. Also, the retrieval of the carrier was found to be more difficult for canals of a larger diameter or taper (Hayakawa *et al.* 2010). The retrievability of the experimental composites should be simpler and easier than the current Thermafil[®] system, however, *in vitro* studies are required to compare the retrievability of these new carrier types with that of the established core-based systems such as Thermafil[®] or GuttaCore.

It is apparent from the tensile test measurements that PE containing 20%HA and 40%HA exhibited a tensile strength lower than that of Thermanfil[®] plastic carriers. There were no significant differences between the composite containing 20% HA and 40% HA. However, the lower tensile strengths of the experimental fibres were high enough to withstand any tensile forces to which the carrier is subjected. The experimental composite with 20% of HA showed a modulus higher than that of 40% hydroxyapatite reinforced composite, and this is indicative that the elastic modulus increased with an increase in the HA content. The composite containing 20% HA with 10% SrO showed a higher flexibility compared with the composite containing 40% HA with 10% SrO. One of the requirements of core materials is an optimum flexibility that will allow the material to follow the curvature of the canals. Although further studies are needed to assess the ability of the experimental core materials to follow the curvatures and to provide a good seal in curved canals, this study indicates that good clinical handling can be achieved. In comparison to the current Flex Point Neo obturator system, comprising of 30% polypropylene with 70% barium sulfate (Flex Point NEO; Neo Dental Chemical Products, Tokyo, Japan), which has also been proposed as a potential carrier material with a temperature tolerance of about 148°C. The current experimental composites are fabricated in this study with a highly biocompatible material. The main difference between HA/PE and Flex Point Neo is in the filler particles as there are no HA particles in the Flex Point Neo. HA low-density PE composites show good potential as carrier materials, and according to preliminary findings, they also show a good adhesion between GP and composite.

The dynamic mechanical behaviour of the hydroxyapatite reinforced low density polyethylene composite fibres at different temperatures was determined by DMA.

The storage modulus, loss modulus, and tan delta data were obtained from DMA. The current study established that the amount of filler particles was a significant parameter in qualifying the storage and loss moduli of elasticity. The properties of any composite material are mainly dependent on the relative quantities and properties of fillers, in terms of particle size, morphology, and size distribution, and matrix as well (Nazhat *et al.* 2000). It was shown by DMA that the storage modulus (elasticity) of HA/PE composites increased with an increase in the quantity of HA filler, whilst the tan delta (viscosity) property of the composite decreased, and thus an inverse relationship between storage modulus and tan delta was recognised. This increase in the storage modulus of the composites may be related to the smaller particle size of HA, 3-5 μ in the polyethylene matrix. Similar findings were reported by several researchers (Nazhat *et al.* 2000), wherein the dynamic mechanical properties of hydroxyapatite reinforced polyethylene were characterised; a significant increase in the storage modulus with an increase in the HA volume fraction at all temperatures and decrease in the storage modulus with an high temperatures for all HA volume fractions were described. The increase in temperatures causes an increase in thermal energy of polyethylene chains making them more mobile and more liable to dissipate energy.

Fluid filtration characterisation

Successful root canal obturation requires the use of materials and techniques capable of obturation the entire root canal system achieving a perfect seal (De Moor & Hommez 2002). Thereby sealing ability tests are important to evaluate the leakage along the canal walls and at the apical portion of the root canal. Thus, the sealing ability of the experimental HA/PE carrier-based obturator

system was compared with that of GuttaCore (GC, Dentsply), when applied in single-rooted extracted human teeth or simulated root canals and the results of this study showed several important features, in particular when these materials were used in combination with a sealer. Indeed, the GC showed lower fluid flow (leakages) than HA/PE during the first 30 min and 60 min of fluid filtration evaluation ($p < 0.05$) when applied in simulated root canals both with and without the use of the sealing agent. However, a continuous reduction of the fluid flow was attained over time with no significant difference in the results obtained at 90 min and in the ultimate leakage value ($p > 0.05$). The same materials applied in human root canals with the use of the sealing agent showed a significantly higher sealing ability ($p < 0.05$) than when applied without sealing agent. The differences in the fluid flow observed in this study were due to the continuous leakages along the root dentine; hence, it was possible to have a quantitative estimation of the voids and gaps along obturator-canal interfaces up to 90 minutes of measurement. Once these leakages were saturated with fluids, then it was possible to assess the ultimate sealing ability of the tested materials. The present outcomes are in accordance with previous fluid filtration studies (Sauro *et al.* 2008, Jalalzadeh *et al.* 2010). Moreover, previous investigations also showed the importance of using a sealing agent along with the root obturation material, in particular, resin-based sealing agents may provide higher sealing ability than glass-ionomer and zinc oxide eugenol sealing agents (De Moor & Hommez 2002). Although, both carrier-based obturation systems presented a comparable sealing ability in human root canals, GC showed in simulated canals a lower leakage filtration than HA/PE in the first 30 min of evaluation. Thus, one may hypothesise that this better sealing ability may have been conferred by the cross-linked gutta-percha in GC which may have better adapted and/or released some

cross-link agents interacting chemically with plastic. Overall, the sealing ability assessment of extracted teeth by fluid filtration of the specifically-designed hydroxyapatite-reinforced polyethylene carrier-based obturator system was comparable to that of GuttaCore.

Voids volume qualification within the root canal space using micro-CT

Because of its high accuracy and non-invasivity, micro-CT technology with a resolution of 6.5 μm was used in this study to locate voids and quantify the volume of voids inside the obturated canal. μCT produced a three-dimensional reconstruction of root canal obturation and its constituents. The null hypothesis was rejected as the percentage of volume of voids of the experimental obturator was comparable to that of commercial one, GC. Although the commercial one showed a lower percentage of voids volume than that of the experimental one, the difference remained non-significant statistically. This could be related to the fact that the experimental obturator was not as tapered as the commercial one. The carriers with tapered design are expected to be well-adapted to the root canal walls. Thus the present study showed that canals obturated with both the experimental obturator and GC exhibited voids within the root canal space.

Measurement of the thickness of gutta-percha coating using micro-CT

Carrier-based techniques are prone to exposure of the carriers during their insertion into the root canal, which would cause the formation of voids between the root canal obturation material and the root canal wall. Stripping of gutta-percha from the carrier has been reported by previous researchers in the apical third (Clark & ElDeeb 1993) and in the middle and apical thirds (Kytridou *et al.* 1999). The present research used a designed model that allowed for the

assessment of the thickness of gutta-percha around the carrier material outside the root canal system using μ CT technology.

Thermafil[®] and GuttaCore carriers are identical in shape and the gutta-percha used for the coating is also identical for the two systems, that is why one of the two carrier types (GuttaCore) was used for the measurement of the thickness of the gutta-percha. μ CT technology is frequently used to assess root canal morphology (Nielsen *et al.* 1995, Dowker *et al.* 1997) and root canal obturation (Jung *et al.* 2005), quantitatively as well as qualitatively. μ CT analysis was also used to evaluate root canal obturation and other dental materials in 3-D reconstructions (Hammad *et al.* 2009, Hatamleh & Watts 2011). In this study the μ CT scanner accurately measured the thickness of gutta-percha around the carriers, wherein the GP thickness of GuttaCore carrier was not uniformly distributed around the carrier. On the other hand, μ CT imaging showed that the thickness of GP on the HA/PE carrier was rather evenly distributed and the carrier was in the centre of GP. It is expected that a uniform thickness of gutta-percha around the carrier would help in achieving more reliable and void-free core-based root canal obturation.

Bondability of gutta-percha coating to the carriers (push-out bond strength)

Adhesion between gutta-percha and a carrier material is an important requirement to help obturate the root canal system with few or no voids. This study attempted to test the bond strength between the GP coating and the carrier of core-based root canal obturation systems using a push-out test. It is worth clarifying two points regarding the geometry of the carrier and their possible effects on the push-out results. The first issue is related to the presence of a groove in Thermafil[®] and GuttaCore carriers. This groove was devised to

facilitate the retrieval of the carriers by use of a file in case of retreatment. The groove is wider in the coronal part than in the middle part of the carrier, and the slices to be subjected to the push-out test were therefore prepared from the middle part of the obturator in order to reduce to a minimum the surface alteration caused by the presence of the groove. In addition, the taper of the carrier in the tested endodontic obturators was 0.04 mm (4%); this taper would not have excessively influenced the results, considering that the tested slices of the carriers were only 1mm-thick. Moreover, the push-out bond strength values were calculated according to the equation estimating the apical taper radius and coronal taper radius to obtain accurate results. The bond between the gutta-percha and the carrier of the experimental obturator was significantly higher than the one obtained with Thermafil® and GuttaCore. This could be due to the micro-mechanical adhesion of the GP to the HA/PE fibres as the fibre-based carriers have irregular surfaces. Similarly, the push-out bond strength of the GuttaCore carrier was found to be significantly higher than that of the Thermafil® carrier. Again the GuttaCore carrier appeared to offer better micromechanical retention than the Thermafil® carrier, since the material used to fabricate the GuttaCore carrier is cross-linked GP and so it is unlikely that any chemical interaction would develop between this and the alpha-phase GP coating. The clinical relevance of the information we gathered on the push-out bond strength of the gutta-percha to the carriers, is however somehow limited, considering that in a clinical context the carriers are heated before insertion into the root canal, and this might result in increased or decreased bond strength of the gutta-percha to the carrier. Further studies are required in order to investigate the push-out bond strength and the quality of the root canal obturation obtained using the most recently developed core-based systems particularly in curved canals. The experimental HA/PE

carrier exhibited the highest push-out bond strength and the new GuttaCore carriers showed a significant improvement in push out strength compared with the Thermafil® ones.

4.5 Conclusion

Newly designed hydroxyapatite reinforced low density polyethylene composites are promising materials for endodontic obturation to function as core carriers for carrier-based root canal obturation. The potential carriers showed better physical (lower melting temperature) and mechanical (flexibility) properties with excellent biocompatibility and easy retrievability in comparison to the current Thermafil® obturator.

Chapter 5 Development & characterisation of low density polyethylene-hydroxyapatite composite as an endodontic obturation core material

5.1 Introduction

This chapter describes the development of a new root canal obturation material and assessment of its feasibility for endodontic use. The newly designed materials were compared with gutta-percha; currently the “gold standard” in root canal obturation.

Obtaining a root canal obturation, free of voids, along with chemo-mechanical preparation of the root canal is important in order to achieve long-term success of a root canal treatment (Ng *et al.* 2011). Gutta-percha is the most widely used root canal obturation material at present (Gutmann & Witherspoon 2002), however there are certain disadvantages such as susceptibility to leakage (Teixeira *et al.* 2004, Shipper *et al.* 2005, Tunga & Bodrumlu 2006), ease of displacement under pressure (Johnson & Kulild 2010), physicochemical instability (Tagger *et al.* 2003, Chandrasekhar *et al.* 2011) and the inability to sterilize it in autoclave or use other conventional sterilization techniques (Stabholz *et al.* 1987) that have adverse effects on the clinical success. The effectiveness of a material to adequately seal the root canal space is governed by its physical properties and handling characteristics.

Gutta-percha has been the preferred root canal obturation material because it possesses many favourable properties, which include biological compatibility, pliability, easy placement and removal, and radiopacity (Weine 1996). Studies have shown that gutta-percha can be adapted to the root canal wall by using various obturation techniques (Collins *et al.* 2006, Natera *et al.* 2011). Despite the

close adaptation, it has been shown that gutta-percha does not create a complete dentinal seal (Tagger *et al.* 2003), with unfilled spaces increasing the potential of coronal microleakage and re-infection of the root canal system, which may contribute to treatment failures (Madison *et al.* 1987, Swanson & Madison 1987). To address this problem, advancements in polymer technology have led to the development of resin-based obturating materials. A polymer obturating material, Resilon™, has been shown to reduce microleakage by effective sealing of the root canal system (Shipper *et al.* 2004). In addition to the aforementioned properties of gutta-percha, Resilon™ has been produced to enhance fracture resistance of endodontically treated teeth and to reduce periapical inflammation after microbial inoculation. On the other hand it has been shown that the polyester component of Resilon™ is subject to a slow degradation process in the presence of moisture (Tay *et al.* 2005b).

In this study we report the synthesis of a low density polyethylene-hydroxyapatite composite to function as a potential material for endodontic use. The rationale for the use of low density polyethylene and hydroxyapatite materials is based on the excellent flexibility and thermoplasticity of LDPE, and tailor-made stiffness and desirable biocompatibility of HA, described in detail in (Section 4.1, Chapter 4). Two sets of composites with differing filler concentrations were produced, one with 20 % silanated HA + 30% SrO, and the other with 30% silanated HA + 20% SrO and labelled as LDPESHASR20/30 and LDPESHASR30/20 respectively. A parallel set of composites using non-silanated HA were also prepared and coded as LDPENSHASRO20/30 and LDPENSHASRO30/20. Previous studies have shown that similar composites could be used successfully as bone analogue materials (Bonfield 1988), that exhibit excellent cytocompatibility.

5.2 Methods

The processing method used to fabricate the experimental composite fibres is described in detail in Section 2.2.1 (chapter 2). The effectiveness of LDPE/HA composite as a potential root canal obturation material was compared with gutta-percha in terms of physical, mechanical and thermal properties.

5.3 Results

5.3.1 FTIR analysis

The infrared spectra of the hydroxyapatite particles after being treated with the silane coupling agent are shown in Figure 4.6 (chapter 4). The FTIR spectral wave range was $4000\text{--}650\text{ cm}^{-1}$. In the spectrum of silanated hydroxyapatite, the absorption band at 1720 cm^{-1} is attributed to the carbonyl group, (C=O) and the peaks at range $1300\text{--}1200\text{ cm}^{-1}$ are ascribed to silicon oxide group, (SiO) respectively, indicating the availability of the coupling agent on the surface of hydroxyapatite. The FTIR spectra of LDPESHASR 20/30, LDPESHASR 30/20 are shown in Figure 5.1. The weak peak at 1370 cm^{-1} and the strong absorption bands between $730\text{--}710\text{ cm}^{-1}$ are assigned to the --CH_2 symmetric deformation and --CH_2 rocking deformation, respectively. A peak at 1465 cm^{-1} emerged due to the bending deformation of the --CH_2 group of the polyethylene. Two sharp peaks at 2848 cm^{-1} and 2916 cm^{-1} were attributed to --CH_2 stretching and --CH_2 deformation of polyethylene, respectively and absorption bands at 962 cm^{-1} and 1022 cm^{-1} , 1091 cm^{-1} are assigned to the phosphate groups, P-O of the hydroxyapatite, indicating the presence of polyethylene matrix and hydroxyapatite filler within the tested composite. The peak intensity of P-O increased with increasing the amount of hydroxyapatite filler. Furthermore, these spectra of FTIR confirm the silanation of hydroxyapatite onto the polyethylene matrix.

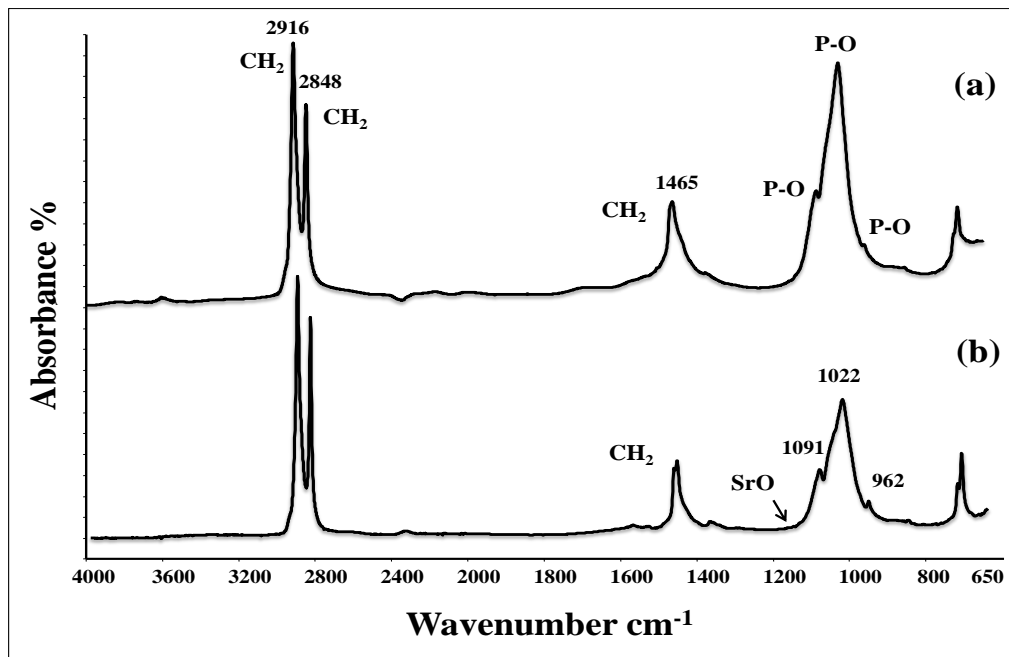


Figure 5.1: FTIR spectra of the a) LDPEHASR30/20, b) LDPEHASR20/30.

5.3.2 Tensile test (tensile strength and elastic modulus)

The tensile strengths and elastic moduli of the silanated HA/PE composites were found to be significantly higher ($P < 0.001$ and $P < 0.0001$, respectively) than that of gutta-percha both when tested under dry conditions and after 1 month storage in simulated body fluid (SBF). The tensile strength and elastic modulus of the non-silanated composites were significantly lower ($p < 0.05$) than those of the silanated composites, (Table 5.1, Figures 5.2 & 5.3). The increase in the weight fraction of hydroxyapatite had a significant effect on the elastic modulus with the composite containing 30% HA by weight exhibiting a higher elastic modulus ($P < 0.05$), however it did not result in a significant change in the tensile strength. A two-way analysis of variance showed significant differences for the elastic modulus ($P > 0.0001$) and tensile strength ($P > 0.001$) of the dry and wet silanated and non-silanated composites but non-significant differences ($P < 0.05$) were observed for the tensile strength of the wet non-silanated composite.

The elastic moduli of the silanated composites of LDPESHASR30/20 and LDPESHASR20/30 were significantly higher ($P<0.001$) than those of gutta-percha both when tested under dry conditions and after 1 month storage in SBF. The tensile strength of these groups did not significantly change after 1 month storage in SBF ($P>0.05$) but they showed a significant change under dry conditions ($P<0.05$). Two-way analysis of variance with Bonferroni's test was performed to statistically analyse the data collection.

Table 5.1: Mean tensile strength and elastic modulus of the hydroxyapatite polyethylene-SrO composites and gutta-percha with standard deviations shown in parentheses (n=6).

	Dry		Wet	
Composites	Tensile strength (MPa) Mean±SD	Elastic modulus (GPa) Mean±SD	Tensile strength (MPa) Mean±SD	Elastic modulus (GPa) Mean±SD
LDPESHASR20/30 ^a	14.21 (1.5)	1.22 (0.07)	14.87 (0.07)	1.17 (0.30)
LDPENSHASR20/30 ^b	11.38 (1.9)	0.99 (0.18)	10.58 (3.10)	0.82 (0.10)
LDPESHASR30/20 ^a	16.29 (0.97)	1.38 (0.1)	19.03 (3.55)	1.40 (0.59)
LDPENSHASR30/20 ^b	11.72 (1.4)	1.17 (0.08)	12.76 (1.67)	1.31 (0.01)
Gutta-percha	7.03 (0.8)	0.074 (0.01)	8.04 (0.86)	0.080 (0.01)

^a Two-way analysis of variance showed significant differences for the elastic modulus ($P>0.0001$) and tensile strength ($P>0.001$) of the dry and wet silanated and non-silanated composites. ^b non-significant difference ($P<0.05$) for the tensile strength of the wet non-silanated composite.

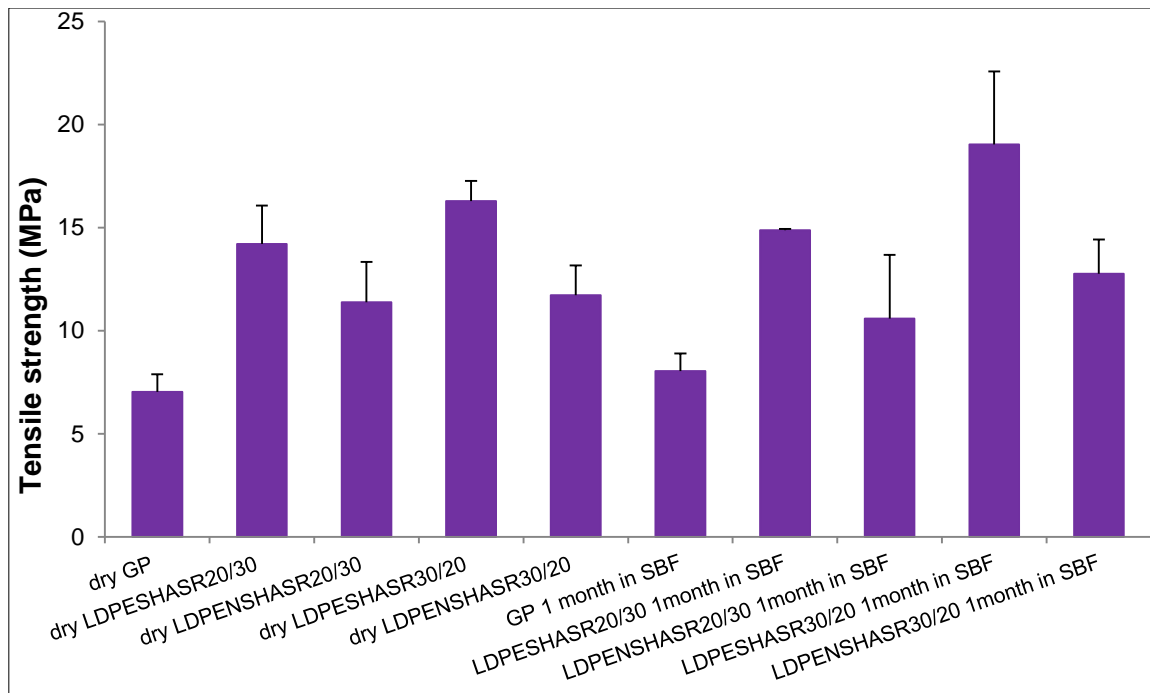


Figure 5.2: Tensile strength graph of the experimental composites and GP under dry and wet conditions.

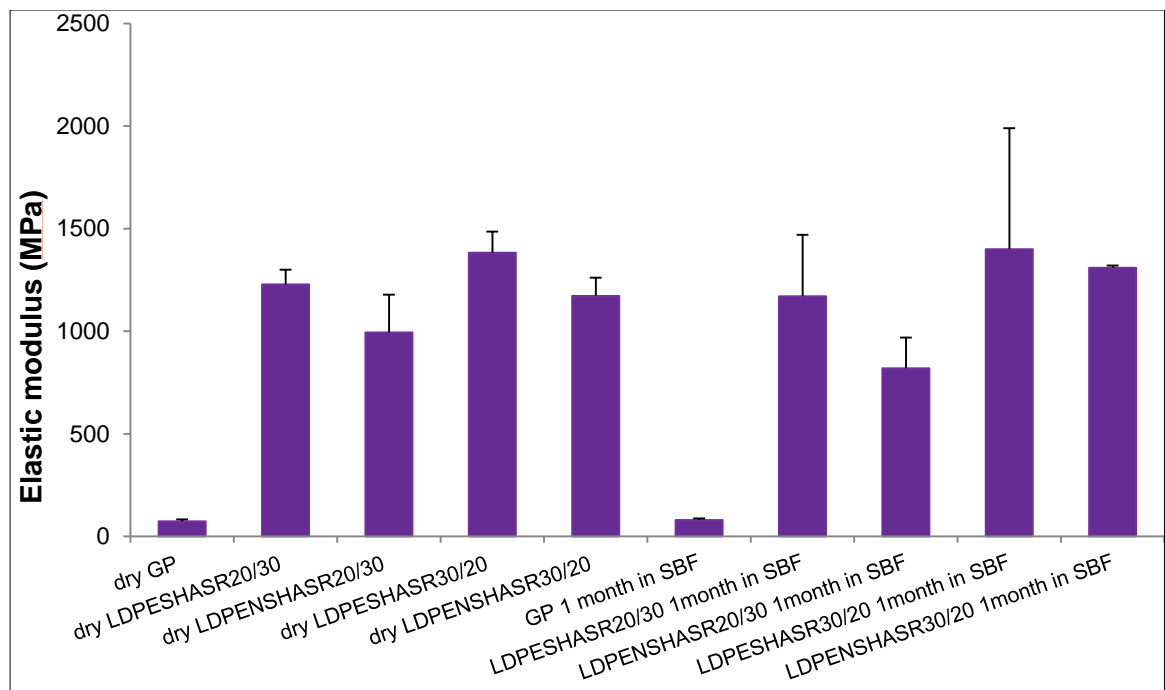


Figure 5.3: Elastic modulus graph of the experimental composites and GP under dry and wet conditions.

5.3.3 DSC analysis

The melting temperatures were determined; the representative thermograms are shown in Figure 5.4. The mean T_m is summarized in Table 5.2. The melting temperature of HA/PE composites ranged between 110.5-111.2°C. It is worth noting that the melting temperatures of the composites with silanated hydroxyapatite remained unchanged. The increase in the weight fraction of hydroxyapatite fillers had no significant effect on the melting temperatures. The melting temperatures of the experimental composites were found to be considerably higher than that of gutta-percha.

Table 5.2: Mean melting temperatures of the HA/PE with SrO and gutta-percha with standard deviations shown in parentheses (n=3).

Composite	$T_m/^\circ\text{C}$
LDPEHASR20/30	110.5 \pm (0.6)
LDPENSHASR20/30	111.0 \pm (1.0)
LDPEHASR30/20	110.8 \pm (0.8)
LDPENSHASR30/20	111.2 \pm (1.2)
GP	52.00 \pm (0.7)

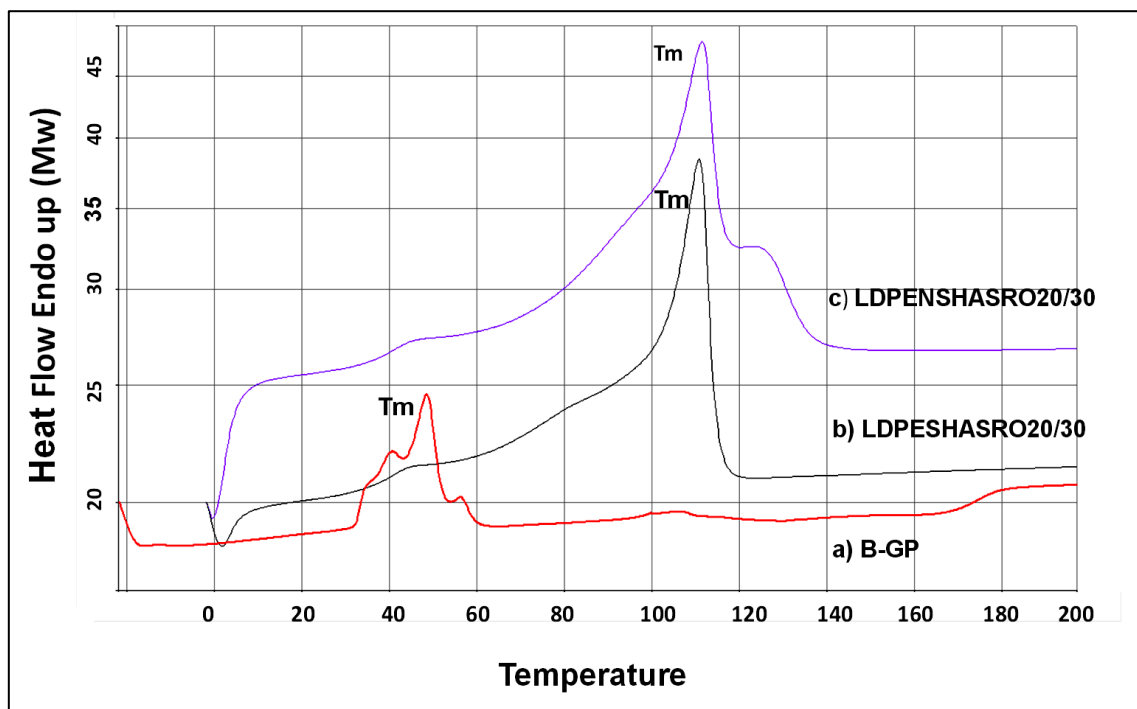


Figure 5.4: Second heating cycles of DSC thermograms showing the melting temperatures of the experimental composites and gutta-percha.

5.3.4 X-ray contrast

The radiographs of the composites of LDPEHASR 20/30 and LDPEHASR 30/20 showed that composites were sufficiently radiopaque, with the degree of radiopacity of LDPEHASR 20/30 being superior to that of LDPEHASR30/20. Radiopacity was expressed in mm of equivalent aluminium and the LDPEHASR20/30 composite showed radiopacity around 4.2 mm when compared with aluminium step wedge steps, estimated by grey-scale values in relation to the thickness (density) of the aluminium as illustrated in Figure 5.5.

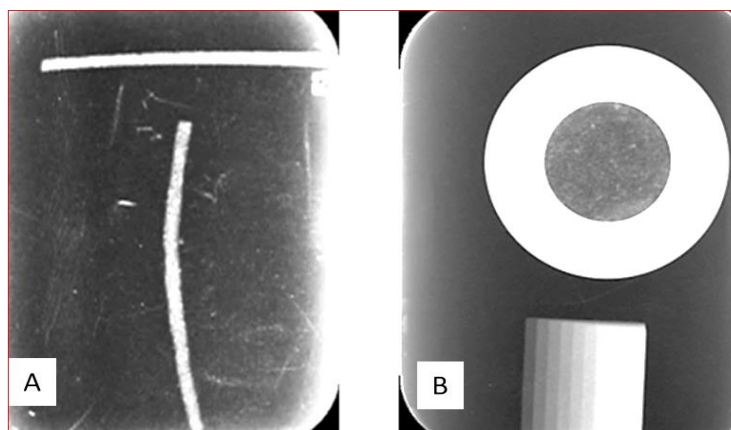


Figure 5.5: Radiograph shows LDPEHASRO20/30 composites (A), and the radiopacity of the composite in the washer is estimated in relation to the thickness of the aluminium steps wedge (B).

Image J software was used to analyse the radiopacity of the test materials in mm of equivalent aluminium step wedge steps. The mean density of the LDPEHASRO20/30 composite condensed in the aluminium washer was 144 which is equivalent to around 4.2 mm Al when compared with steps of aluminium step wedge as shown in Figure 5.6.

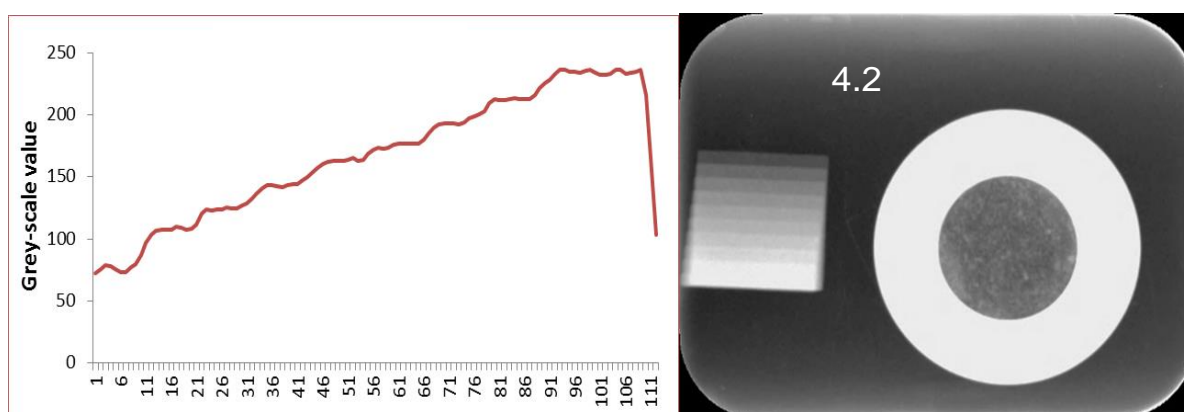


Figure 5.6: Radiograph showing the radiopacity of the LDPEHASRO20/30 composite in the washer estimated in relation to the density of the aluminium steps wedge using image J software plot profile analysis (n=5).

However, the mean density of the LDPESHASRO30/20 composite was 112, expressed about 3.9 mm Al degree equivalents to the aluminium step wedge steps as seen in Figure 5.7.

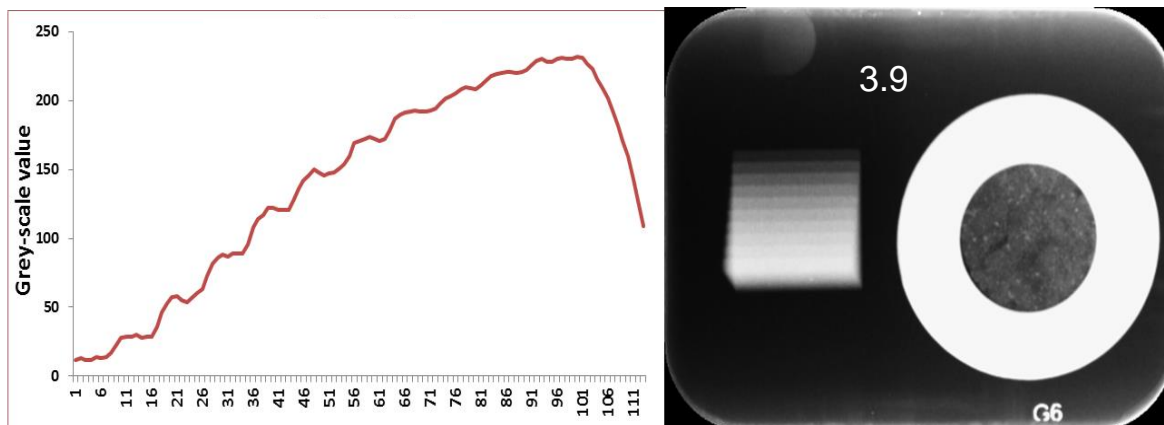


Figure 5.7: Radiograph showing the radiopacity of the LDPESHASRO30/20 composite in the washer estimated in relation to the density of the aluminium steps wedge using image J software plot profile analysis (n=5).

The experimental composites showed considerable flexibility and subjectively had excellent handling. The HA-LDPE composite fibres were able to bend to an angle of 90 degree or greater, whereas the gutta-percha fractured or even cracked when bent around 90 degree as seen in Figure 5.8, indicating the fibres were able to maintain their integrity on manipulation, which may often be incurred during clinical placement.

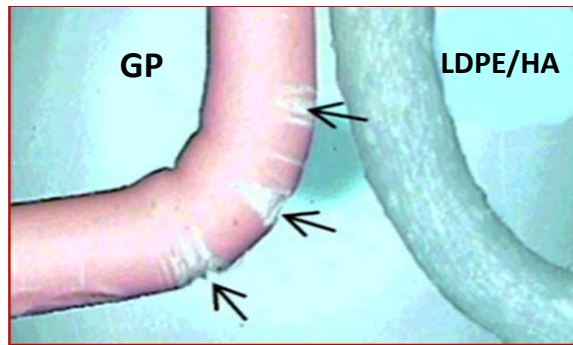


Figure 5.8: Image showing fractured gutta-percha compared with intact experimental composite (LDPE/HA) when bent 90 degree or less (*arrows*); (magnification X 2).

5.3.5 Interaction of the test materials with Eugenol

The main rationale for characterising the interaction of the test materials with eugenol is that eugenol is one of the main components of zinc oxide eugenol root canal sealers, which are commonly used in root canal obturation in combination with the obturation core material (i.e. gutta-percha); the zinc oxide eugenol sealer was described in detail in Section 1.3.5.1 (Chapter 1). Five weighed cones (20 mm-long) of silanated composite fibres, non-silanated composite fibres and gutta-percha were immersed in eugenol. The interaction of the composites and gutta-percha with eugenol was assessed by measuring gravimetric changes after 1, 2 and 5 days of immersion in eugenol. The results of the interaction test showed that the weight of LDPE/HA composite in eugenol did not exhibit significant changes after 1, 2 and 5 days ($P > 0.05$) whereas, gutta-percha showed a significant increase in the weight particularly after 5 days ($P < 0.001$) as shown in Figure 5.9.

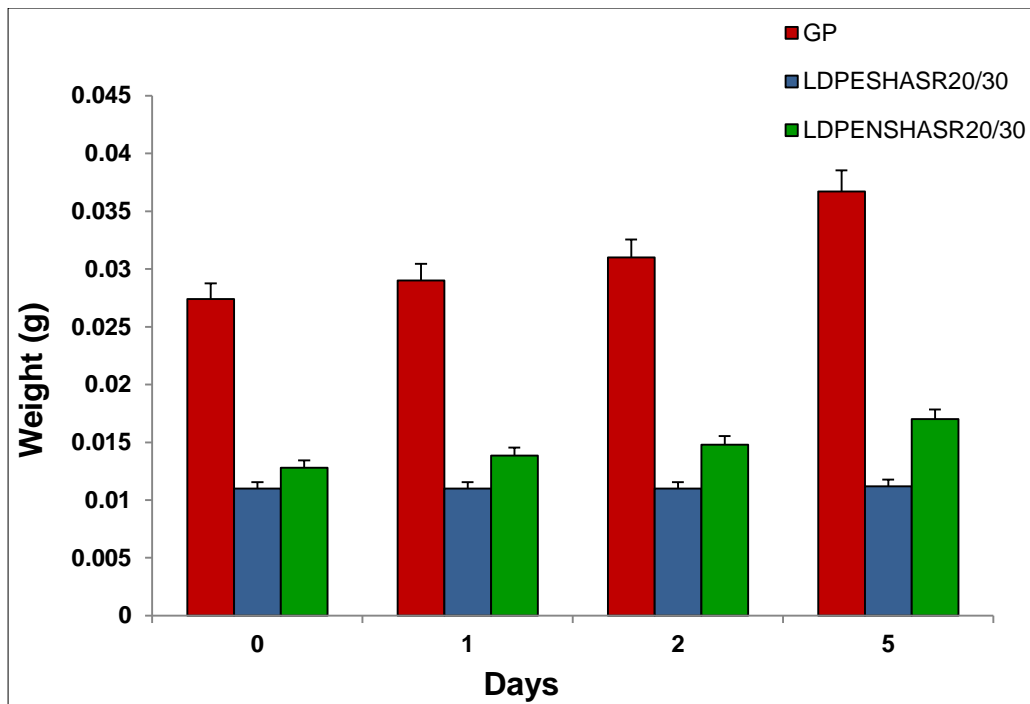


Figure 5.9: Interaction of the experimental materials and gutta-percha with eugenol (n=5).

5.3.6 Physical integrity of the experimental composites after sterilisation with gamma irradiation

The polyethylene containing hydroxyapatite composites are sterilisable by gamma radiation. The length, weight and thickness of the fibres were measured before and after gamma irradiation. The findings of sterilisation test showed non-significant differences suggesting that gamma sterilisation was an effective method to disinfect the composite fibres. The sterilisation test results are shown in Table 5.3.

Table 5.3: LDPE/SHA with SrO composite masses before and after gamma sterilisation (n=5).

Composites	Wt before gamma radiation (g)	Wt after gamma radiation (g)	Length before gamma radiation (mm)	Length after gamma radiation (mm)	Thickness before radiation (mm)	Thickne ss after radiation (mm)
LDPESHAS R20	0.0201	0.0201	25.00	25.00	1.00	1.00
LDPESHAS R30	0.0201	0.0201	25.00	25.00	1.00	1.00

5.3.7 Intra-canal temperature measurements

The temperature inside the root canal space was measured using a thermocouple system, after obturation of root canals using a system B heat source to thermoplasticize and condense the gutta-percha into the apical region of root canal space. System B was used at 200°C. The results of the experimental composites showed non-significant changes ($P>0.05$) in the recorded temperatures within the root canal space compared with the gutta-percha, particularly in the apical regions. These findings suggest that the experimental composites can be safely used to obturate the canal space with System B without causing undesirable complications on the adjacent tissues. The intra-canal temperatures recorded at each level within the root canal are presented in Table 5.4 & Table 5.5. A linear increase in the mean temperatures was observed inside the root canal from the apex to the coronal opening.

Table 5.4: Mean intra-canal temperatures (°C) produced using System B for the condensation of gutta-percha and PE/HA

Thermocouple channels	*Thermocouple level	Mean temperature of PE/HA /°C	Mean temperature of Gutta-percha /°C
Channel 1	1	27.9	27.6
Channel 2	4	28.2	28.2
Channel 3	7	29.2	29.5
Channel 4	10	30.5	31.7
Channel 5	13	34.1	43.3

*thermocouple level in mm from anatomical root apex

Table 5.5: Maximum and minimum Intracanal temperatures (°C) produced using System B for the condensation of gutta-percha and PE/HA.

Thermocouple channels	*Thermo couple level	Maximum temperature of PE/HA /°C	Minimum temperature of PE/HA /°C	Maximum temperature of gutta-percha /°C	Minimum temperature of gutta-percha /°C
Channel 1	1	28.1	27.8	28.5	26.8
Channel 2	4	28.5	27.7	29.8	26.7
Channel 3	7	30	28.5	31	28
Channel 4	10	31	29.5	34.1	29.3
Channel 5	13	39.2	29	52	34.6

*thermocouple level in mm from anatomical root apex.

5.3.8 Biocompatibility study

5.3.8.1 MTT assay

The results from the MTT assay showed that LDPEHA composites exhibited no deleterious effects ($P > 0.05$) on the metabolic activity of cells at 1 day and 3 day exposure compared to the negative non-toxic control group Figure 5.10 & 5.11. The concentration of the filler and the radiopacifier did not exhibit any significant influence on the eluants. Cell exposed to eluants from the test materials showed a non-significant drop in metabolic activity at 72-h (green bar) following 1 day cell exposure as shown in Figure 5.10.

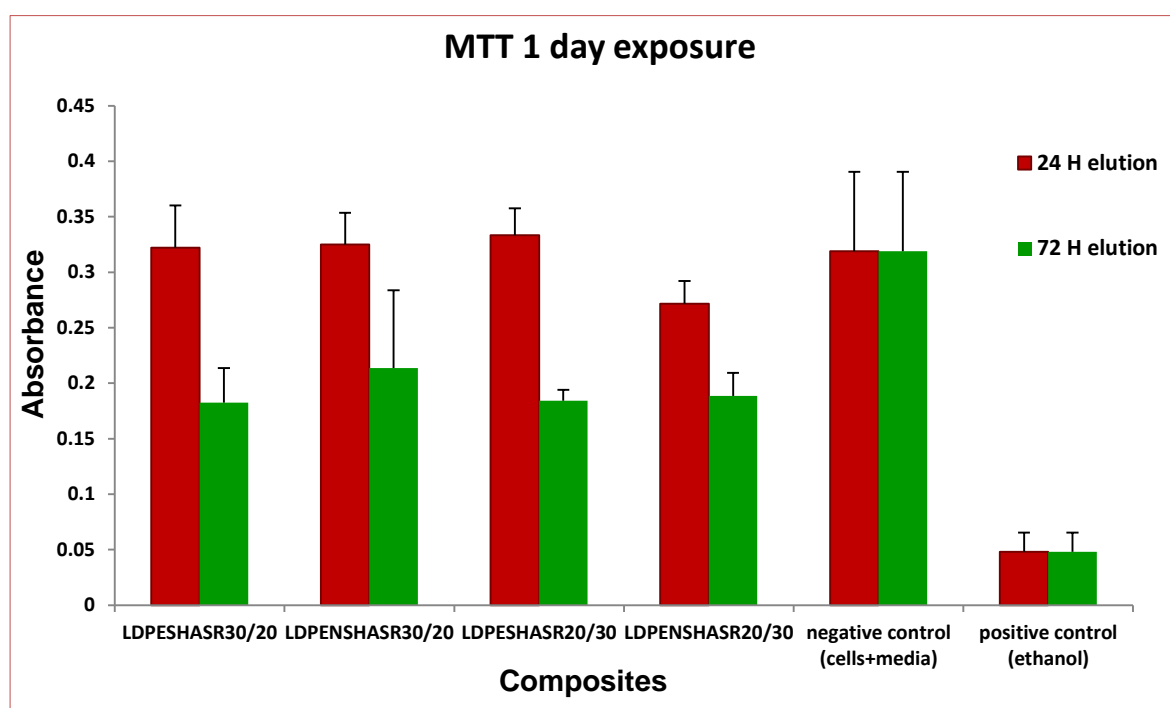


Figure 5.10: Viability of human osteoblast-like cells following exposure for 1 day detected at 24-h and 72-h eluted media from the experimental composites, expressed as mean & SD absorbance.

At 3 day cell exposure, there was an improvement in the metabolic activity of the cells detected at 24-h and 72-h elution fluids compared with the non-toxic control

group, indicating a good biocompatibility particularly at 24-h elution after 3 day exposure as seen in Figure 5.11.

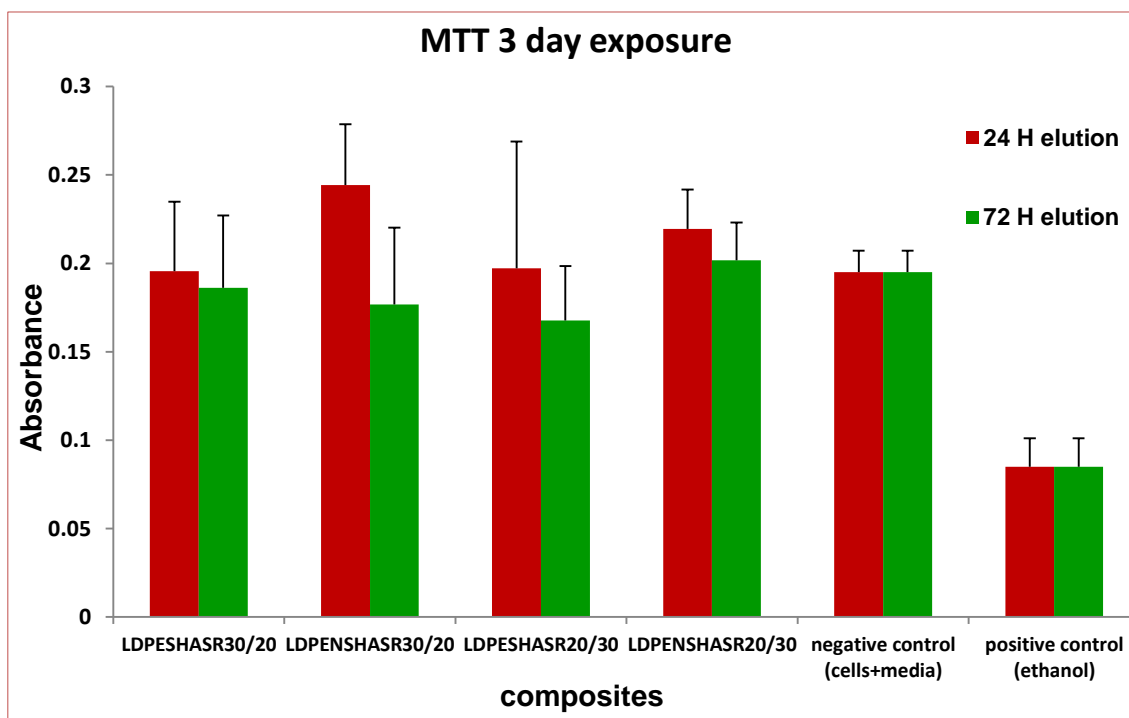


Figure 5.11: Viability of human osteoblast-like cells following exposure for 3 day detected at 24-h and 72-h eluted media from the experimental composites, expressed as mean & SD absorbance.

5.3.8.2 alamarBlue™ assay

The results for the cell proliferation (direct study) on the test materials for 28 days compared with TMX (control) are shown in Figure 5.12. alamarBlue™ assay results indicated that cell proliferation on the LDPEHA composites showed no significant difference ($P > 0.05$) in comparison to the non-toxic control Thermanox™ during the first 7 days. However, between the 14th and the 28th day the composites containing higher concentration of hydroxyapatite showed a significantly higher cell proliferation ($P < 0.001$) compared to Thermanox™ (TMX).

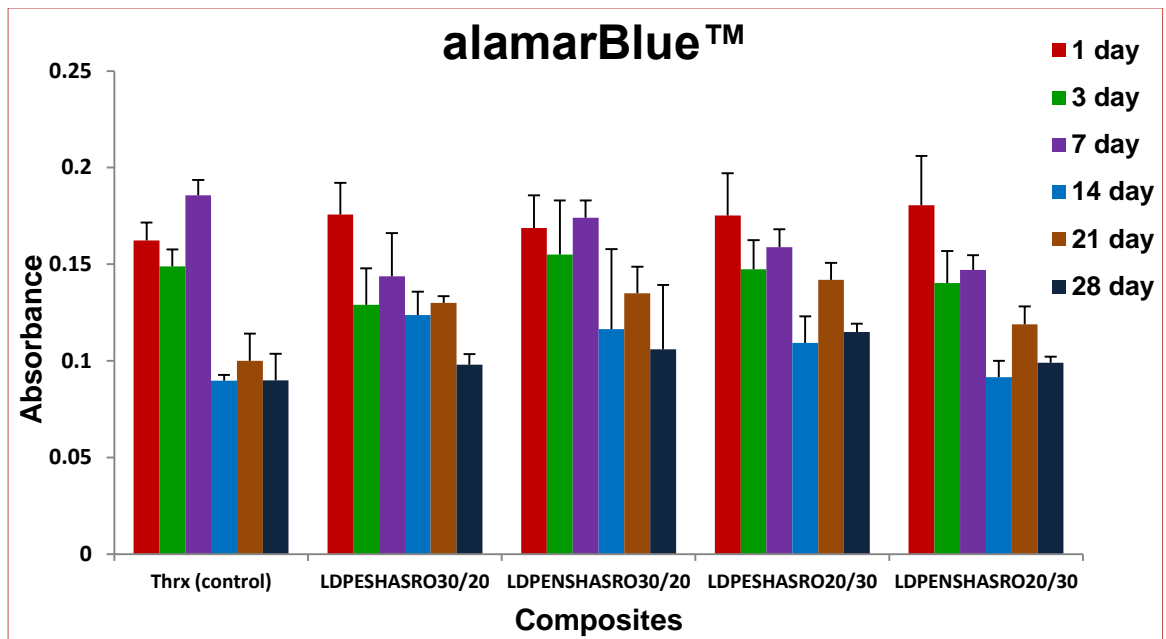


Figure 5.12: Human osteoblast-like cell proliferations as indicated by alamarBlue™ assay in direct contact with the experimental composites composite at 1, 3, 7, 14, 21 and 28 days.

5.3.9 Interaction of the composites with simulated body fluid at 37°C

5.3.9.1 LDPESHASRO

Overall, there were no measurable changes in the weight of the investigated composites. The weight of the fibres remained unchanged over the 49 days of immersion in SBF as shown in Figure 5.13. These findings indicate that the silanated fibres were stable in SBF over the test period due to a good interaction between the silanated HA and the polymer matrix at their interface. The immersion of the fibres in SBF is also an accelerated test since fibres placed in the root canal are not exposed to fluids and are mainly dry.

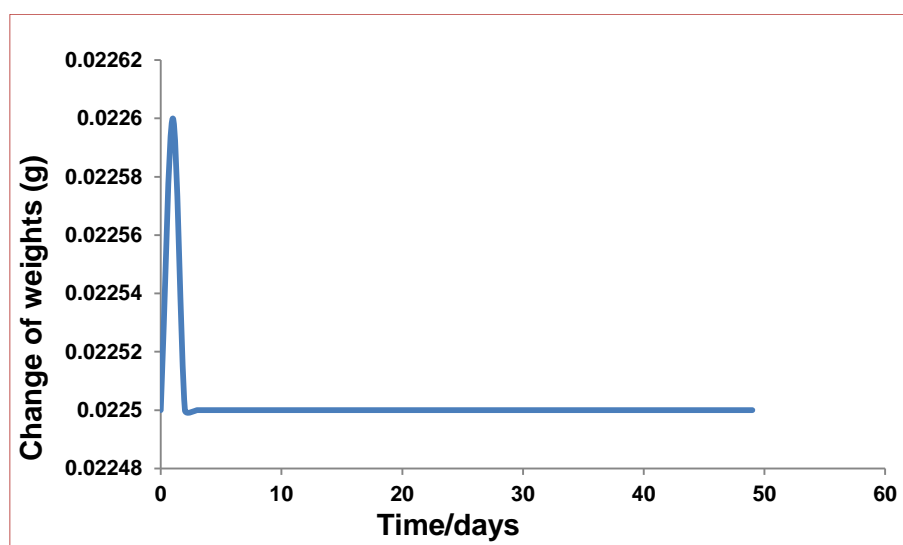


Figure 5.13: A plot of weight (g) against time (days) of LDPESHASRO20/30 in SBF.

5.3.9.2 LDPENSHASRO

There was a slight increase in the weight of fibres in the first and second day of the experiment as shown in Figure 5.14. Between 7 to 14 days, the weight of fibres returned to its original weight. The weight of fibres exhibited a gradual decrease after 14 days reaching 0.0056g at 35 days and keeping the same weight at 49 days, when the experiment was terminated. These results suggest that non-silanated fibres are susceptible to decompose in SBF because of the interaction between non-silanated HA and polymer matrix.

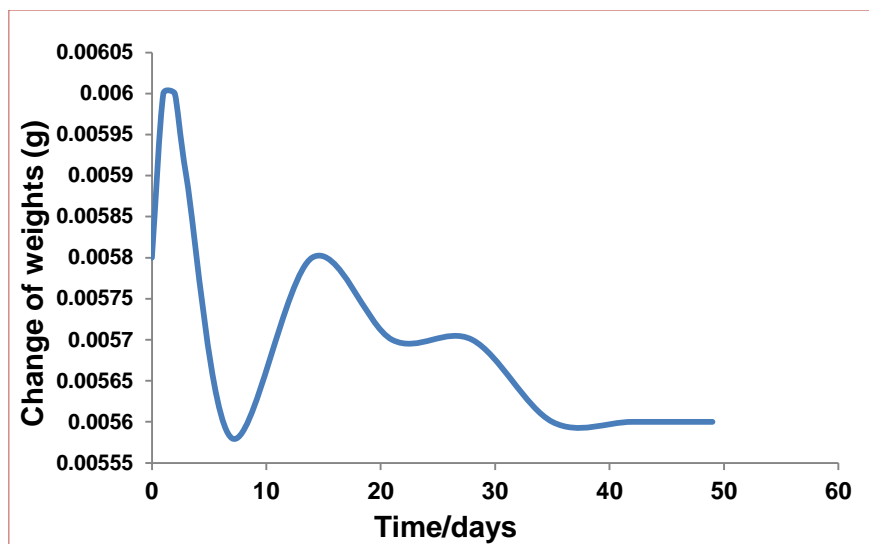


Figure 5.14: A plot of weight (g) against time (days) of LDPENSHASR20/30 in SBF.

5.3.10 Scanning Electron Microscopy (SEM)

In order to examine the dispersion and distribution of hydroxyapatite particles in polyethylene resin matrix, SEMs were undertaken. The SEM micrographs of the experimental composites (LDPESHASRO30/20 & LDPESHASRO20/30) showed a homogenous structure in the bulk of the fibres with variable and inconsistent amounts of white deposits of SrO and HA filler particles. The micrographs are shown in Figure 5.15. It is apparent from SEM that a good dispersion and uniform distribution of hydroxyapatite particles in polyethylene matrix in the composite was observed. Thus, improvement in the mechanical and biological properties of the composite is expected.

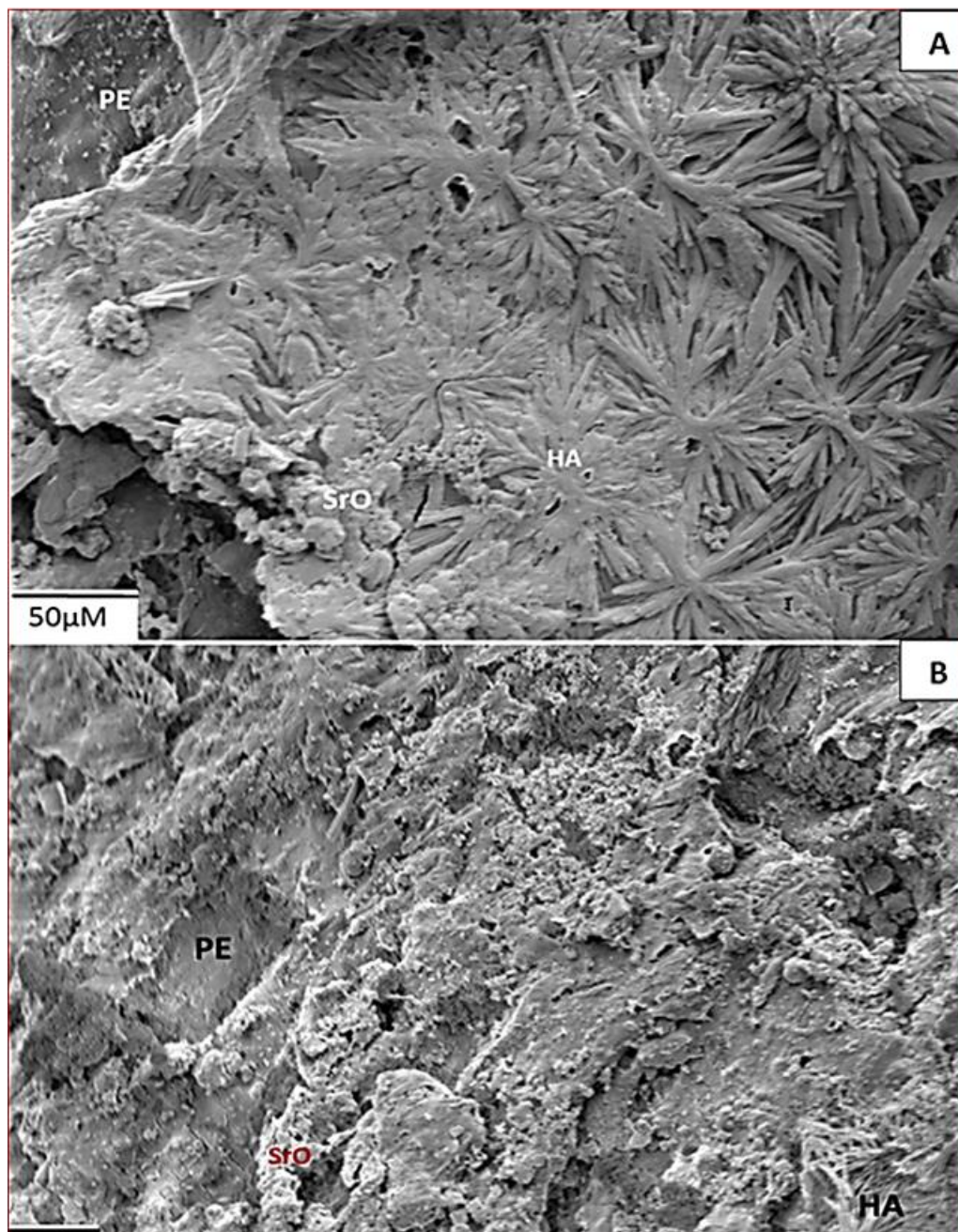


Figure 5.15: SEM micrographs (500 x magnifications) of the experimental composites, A) LDPESHASRO30/20 & B) LDPESHASRO20/30).

5.3.11 Discussion

Endodontic obturation materials aim to promote a complete seal in a region of complex anatomy. Endodontically treated teeth are also exposed to occlusal forces, which stress the interface between the obturation material and the root canal wall thereby compromising the seal (Bishop *et al.* 2008). The overall procedure of root canal treatment weakens the strength of the tooth structure and makes it at a greater risk of fracture (Lam *et al.* 2005). This fact has led to some clinicians to reinforce the remaining tooth structure via using adhesive root canal obturating materials (Teixeira *et al.* 2004). However, the clinical data that support this beneficial adhesive response are limited (Stuart *et al.* 2006, Williams *et al.* 2006). Several investigators have suggested that the obturation material with stiffness approaching that of dentine could help strengthening the endodontically treated tooth and lowering the risk of root fracture and thereby improving the outcome of root canal treatment (Williams *et al.* 2006, Grande *et al.* 2007). Thus, the experimental composites of hydroxyapatite reinforced low density polyethylene developed in the current work have potential to reinforce the endodontically treated root due to the high stiffness in comparison to gutta-percha. Matrix composition, filler type, size and size distribution of filler particles, quality of interfacial bonding and the amount of filler can all affect the physical and mechanical properties of composites (Labella *et al.* 1994). In this study the filler used was HA. The filler content was limited to 20% and 30% by weight of HA with the addition of SrO to impart radiopacity to the material. The net low density polyethylene matrix content was maintained at 50% by weight in both experimental composites with 20% and 30% by weight of SrO. FTIR spectra were recorded to confirm the components of the experimental composites tested in the present study. The main bands of LDPE were at 718 cm^{-1} , 1468 cm^{-1} , 2849 cm^{-1} , and 2916 cm^{-1} . This observation is in agreement with the findings of a previous

study (Santhoskumar *et al.* 2010). New peaks, particularly at 1033 cm^{-1} and 1758 cm^{-1} , were observed in the infrared spectra of LDPE by adding HA fillers and SrO. The tensile strength and elastic modulus (stiffness) of HA/LDPE composites were significantly more favourable than gutta-percha both when tested under dry condition and after 1 month storage in SBF. The amount of HA filler in the composites is of paramount importance in tailoring the degree of flexibility of the composite. In the current study, the elastic modulus of composites significantly increased with an increase in the weight fraction of HA particles. Enhancement of the mechanical properties of polyethylene with the incorporation of HA has also been verified by several investigators, wherein inclusion of HA particles into high density polyethylene improved the stiffness of the composite to function as bone analogue and implant materials (Bonfield *et al.* 1998, Tanner 2010).

Zinc oxide eugenol root sealant material is often used in the root canal obturation in combination with the obturating material and thus the interaction of eugenol with the obturating materials was investigated in the current study. As the reaction of zinc oxide-eugenol occurs over variable periods of time, it is possible that the obturating material may come in contact with free eugenol (Prosser & Wilson 1982, Camps *et al.* 2004). The physical interaction of LDPESHASR 20/30, LDPENSHASR 20/30 composites, and gutta-percha were characterised after 1, 2, 5 days of immersion in eugenol. The results obtained from the physical interaction showed that LDPESHASR (silanated) composite exhibited good stability with no evidence of weight change determined after immersion in eugenol whereas significant expansion of gutta-percha and LDPENSHASR20/30 (non-silanated) was observed. The expansion of gutta-percha may be attributed to the ionic reaction of eugenol with zinc oxide filler of gutta-percha, and thus the distortion and swelling of gutta-percha point may cause decomposition of the

trans-polyisoprene polymer of gutta-percha, which produces voids within the root canal obturation itself that could be occupied by bacterial biofilms and thus, failure of treatment. Volumetric expansions of gutta-percha in contact with eugenol containing sealers have been reported by several investigators (Michaud *et al.* 2008, Chandrasekhar *et al.* 2011). The current physical interaction study with eugenol confirmed the beneficial response of silanated versus non-silanated composites in achieving a more stable and durable composite. Gutta-percha, the most commonly used root canal obturation material, easily deteriorates on sterilisation or chemical disinfection, which may be a disadvantage. The integrity of polyethylene-hydroxyapatite composites was also examined and compared with gutta-percha using optical microscope when they bent 90 degree or less; the investigations of the experimental composites showed an adequate amount of flexibility, which is required for the obturating materials to be easily inserted within the root canal space and no evidence of fatigue was observed, whereas crack or even fractures evidence were noticed in gutta-percha. This is an advantage of the experimental composites over gutta-percha.

The melting temperatures of hydroxyapatite reinforced polyethylene composites were characterised using DSC. It is apparent from the results of DSC measurements that the melting temperatures were in the region of 110-111°C, which are significantly higher ($P < 0.05$) than gutta-percha (around 52-59°C). This is clinically relevant for removal of the experimental composites using heat application in cases of retreatment. Several investigators have reported that gutta-percha melted at around 52-59°C using DSC (Combe *et al.* 2001) which supports the current results obtained from DSC in this study. Literature values reported for the melting temperature of Resilon™, a relatively new root canal

obturation material ranged from 60-70°C, which is also lower than the experimental composites.

The intra-canal temperatures of the gutta-percha and experimental composites were recorded using a thermocouple technique for canals obturated using System B heat obturation. Non-significant differences were observed between gutta-percha and experimental composites in terms of the intra-canal temperatures recorded. A gradual and linear increase in the temperature from the apical level towards the coronal level was also reported. This finding could be related to the bigger volume of the obturation material in the most coronal part compared with the apical part. These observations are consistent with the study of Weller *et al* (1991), wherein a thermocouple model was developed to measure intracanal temperatures produced by high-temperature thermoplasticized injectable gutta-percha. A linear increase in the recorded temperatures in the root canal was found. Furthermore, the SrO, incorporated in the composite not only imparted radiopacity to the material but also may exert an antibacterial action and may potentially help in eliminating bacteria from the root canal space (Guida *et al.* 2003), however this needs further investigation.

The radiopacity of the LDPESHASR 20/30 and LDPESHASR 30/20 composites were assessed against an aluminium step wedge using digital radiography and storage-phosphor systems. The results of the X-rays were analysed using image J software. It has been suggested by the standards founded by (ANSI/ADA 2000) Specification No. 57, that the obturation materials are considered radiopaque when they possess a radiopacity not less than that equivalent to 3 mm of aluminium. The current study reported that the experimental composites of HA reinforced LDPE showed requisite radiopacity that allow for a clear distinction between the material and the surrounding tooth structure. Thus, the radiopacity of

LDPESHASR20/30 was found to be superior to LDPESHASR30/20 due to the higher amount of SrO present in the LDPESHASR20/30, indicating that the SrO content enhances the radiopacity.

The biological response of the experimental HA reinforced LDPE on primary human osteoblast-like cell was characterised using *in vitro* cell culture studies represented by MTT and alamarBlue™ assays. The MTT assay reported no cytotoxic effects on the viability and metabolic functions of osteoblast-like cells after 1 day and 3 day cell exposure, indicating a good biological response with vital tissue and no leachable toxic ions released. The initial contrast of increasing (24-hour elution) and lowering (72-hour elution) of MTT activity at 1 day exposure expressed on cells may be related to the changes in the pH of a hydroxyapatite reinforced polyethylene composite because of the interaction of the culture medium with hydroxyapatite from the composite, which may cause the release of ions present in the ceramic. At day 3 of the MTT assay, a significant increase in the cellular metabolic activity compared with the tissue-plastic culture control was observed, suggesting that hydroxyapatite in the composite had the ability to provide favourable sites for cell adhesion and growth. This may be attributed to the release of calcium and phosphate ions which are vital for bone formation. The alamarBlue™ test showed an enhanced proliferation of osteoblast cells after 14 days of cell culture compared with the control (TMX). This enhancement of cell proliferation may be accounted for the presence of HA crystals in the composites which elicit suitable sites for cell attachment. The further justification of the enhanced cellular behaviour may be the irregularity of the surface of HA reinforced LDPE composites. The biocompatibility data reported in the present study were supported by previous studies, wherein the biocompatibility of HA reinforced HDPE composites has been reported (Di Silvio *et al.* 2002, Zhang *et*

al. 2007, Zhang *et al.* 2009c). It is also interesting to note that the inclusion of SrO into the composite produced no significant changes on the metabolic activity and growth of cells. A further beneficial response of the silanated HA reinforced LDPE composites over the non-silanated materials was observed in the current study when the integrity of the composites (silanated and non-silanated) was tested after immersion in SBF for 49 days at 37°C. It was apparent from the physical integrity test that the silanated composite remained unchanged with no evidence of weight loss over the 49 days of immersion in SBF, whereas the non-silanated composite showed evidence of weight loss over the same period. This finding is attributed to the effect of the use of the silane coupling agent on the composite, establishing a more stable and durable composite.

The dispersion and distribution of hydroxyapatite fillers in the low density polyethylene matrix was examined using scanning electron microscopy. It is apparent from the scanning electron microscopy that a good dispersion and homogenous distribution of hydroxyapatite particles in polyethylene resin matrix was achieved. This distribution of filler could help in improving the biological and mechanical properties of the composite.

5.3.12 Conclusion

Within the limitations of this study, it could be concluded that the silanated HA reinforced LDPE with SrO composites in particular, LDPESHASR 20/30 composite, are promising materials for endodontic obturation based on their elastic modulus, tensile strength (stiffness), and radiopacity. The experimental composites are potentially able to reinforce the endodontically treated root; they can be heat-sterilised and they are easily removed from the root canal and show excellent biocompatibility.

Chapter 6 Development & characterisation of low density polyethylene-Bioglass[®] composite as an endodontic obturation core material

6.1 Introduction

The applications of composites with biodegradable or non-degradable polymeric matrices and bioactive ceramic fillers for biomedical applications are well established (Rezwan *et al.* 2006, Skrtic & Antonucci 2011). Furthermore, the ability to tailor properties such as water uptake, stiffness, toughness, biocompatibility and thermal properties provide a range of design parameters to suit the requirement of the intended application.

In the field of tissue engineering, a widely used strategy for repair and regeneration is the use of composite scaffolds to guide tissues and provide mechanical support for their growth. Hence a large number of polymer-ceramic composite materials have been used as scaffolds, specifically for bone tissue engineering.

A bioactive glass, 45S5 Bioglass[®] has the ability to form a hydroxycarbonate apatite layer (HCA) when immersed in physiological fluid, a layer that is similar to the bone mineral component. Bioactive ceramics produce a unique biological response between the material itself and the adjacent tissue forming a bioactive bond. The bond is established on the material surface via the nucleation and growth of an apatite-like phase, creating material-tissue integration (Arcos *et al.* 2009). Thus, 45S5 Bioglass[®] reinforced polymer composites have been reported for several biomedical applications and particularly Bioglass[®] reinforced polyethylene composites have been reported to combine the high bioactivity of

Bioglass[®] and toughness of polyethylene. Additionally, 45S5 Bioglass[®] composites are able to impart antibacterial properties even when embedded in a polymeric composite, due to the high alkalinity of the glass phase (Koller *et al.* 2008). Thus, a reinforced biocompatible composites, with a biostable polymer such as polyethylene, have the potential to be developed as a root canal obturation material, especially if an antibacterial effect is elicited, which may be beneficial in the root canal space.

In this chapter the development and characterisation of composites of low density polyethylene with 45S5 Bioglass[®], using a melt-extrusion process, as potential root canal obturation materials, are reported. Two sets of composites with differing filler concentrations were produced, one with 20 % Bioglass[®] + 30% SrO, and the other with 30% Bioglass[®] + 20% SrO and labelled as LDPEBAGSR20/30 and LDPEBAGSR30/20 respectively.

6.2 Methods

The processing method used for development of polyethylene-Bioglass[®] composites was similar to that of the polyethylene-hydroxyapatite composite described in Chapter 2. Composites consisting of low density polyethylene-Bioglass[®] /SrO were prepared and characterised. As the total inorganic content was maintained constant; thus, extrusion parameters required no changes in the processing parameters of the two different composites.

6.3 Results

The composite fibres were obtained using a temperature of 150°C in the barrel of the extruder with a die temperature of 160°C. The average particle size of the 45S5 Bioglass[®] was 45-80 µm with a mean diameter of 45 µm. The extruded composites showed no signs of visual discolouration. The composites were

characterised and the interaction with simulated fluid tested in order to confirm that the composites had the Bioglass[®] exposed on the surface. The presence of the Bioglass[®] on the surface can be related to possible antibacterial activity, however this is not conclusive and direct zone of inhibition tests are required.

6.3.1 FTIR analysis

The FTIR spectra of the LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites before and after 2 days soaking in SBF were obtained to characterise the apatite-like layer formation on the surface of the composites. The FTIR spectral wave range was 4000-650 cm⁻¹. In the spectrum of both LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites before soaking in SBF, the band at 1465 cm⁻¹ which is ascribed to the stretching vibration of CH₂ and two sharp peaks at 2848 cm⁻¹ and 2916 cm⁻¹ are due to -CH₂ stretching and -CH₂ deformation, which originate from the polyethylene segments, the absorption bands at range of 1040-900 cm⁻¹ are attributed to the stretching vibrations of Si-O, confirming the presence of Bioglass[®], as shown in Figure 6.1A & Figure 6.2A. In the spectrum of LDPEBAGSRO 20/30 composite after soaking in SBF for periods of up to 2 days at 37°C, the Si-O vibration mode disappeared and a new broad phosphate peak, P-O emerged at 1037 cm⁻¹. The formation of this peak is related to the calcium phosphate precipitation suggesting that an amorphous calcium phosphate layer is beginning to form.

A small peak at 871 cm⁻¹, due to the carbonate was also observed. Another broad peak of carbonate also appeared at 1621 cm⁻¹, indicating the incorporation of carbonate groups in the structure of apatite forming hydroxycarbonate apatite-like layer on the surface of composite as shown in Figure 6.1B & Figure 6.2B. Thus, using FTIR, there is evidence of P-O and carbonate absorption bands, indicating that a hydroxycarbonate apatite-like layer formed on the surface of the

material. CH_2 vibration peaks were also observed in the region of $1600\text{--}1400\text{ cm}^{-1}$, which were superimposed with other carbonate peaks in the same region, thus making it difficult to confirm that the peak was related to carbonate or CH_2 vibration modes as shown in Figure 6.1 & Figure 6.2.

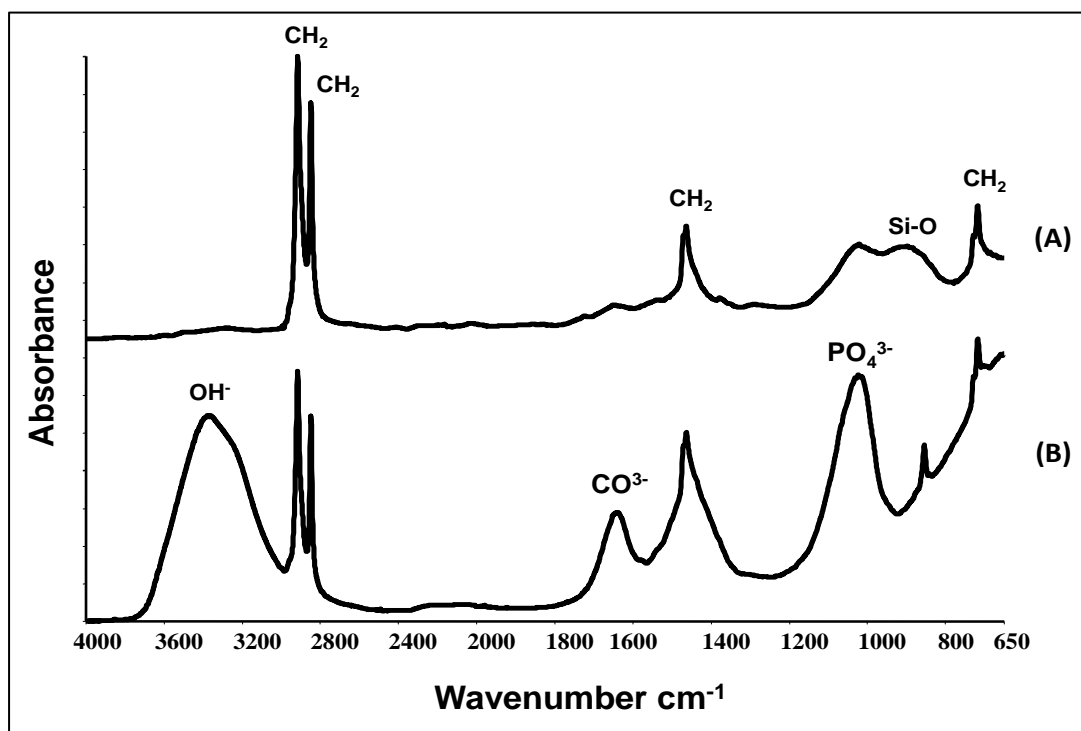


Figure 6.1: FTIR spectra of LDPEBAGSRO 20/30 composite (A), and LDPEBAGSRO 20/30 after 2 days immersion in SBF (B).

Whilst in the spectrum of LDPEBAGSRO 30/20 composite after immersion in SBF, the FTIR spectra exhibited similar peaks to LDPEBAGSRO 20/30 in terms of availability of PO_4 and CO_3 peaks as shown in Figure 6.1b & Figure 6.2b, the main difference was related to the intensity of the phosphate peak, P-O which was slightly higher than that of LDPEBAGSRO 20/30 due to the presence of a higher amount of Bioglass 30%.

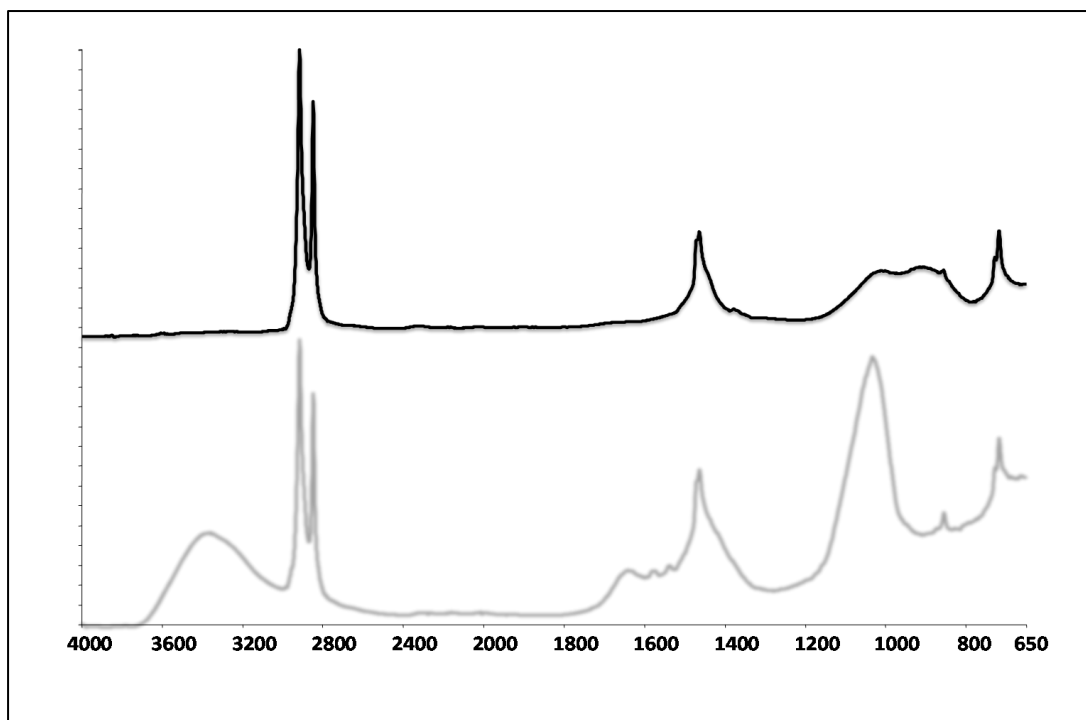


Figure 6.2: FTIR spectra of LDPEBAGSRO 30/20 composite (A), and LDPEBAGSRO 30/20 after being immersed 2 days in SBF (B).

6.3.2 Tensile test (tensile strength and elastic modulus)

The tensile strength and elastic moduli of LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites were found to be significantly higher ($P < 0.05$ and $P < 0.0001$, respectively) than that of gutta-percha both when tested under dry conditions and after 1 month storage in simulated body fluid (SBF). The results of elastic moduli and tensile strength of polyethylene-Bioglass[®] composites are shown in Figure 6.3, 6.4 and summarized in Table 6.1. Comparing polyethylene-hydroxyapatite composites with polyethylene-Bioglass[®] composites, the tensile strength and elastic modulus of polyethylene-hydroxyapatite composites were significantly higher ($P < 0.05$) than those of the polyethylene-Bioglass[®] composites when tested under dry conditions and after 1 month storage in SBF.

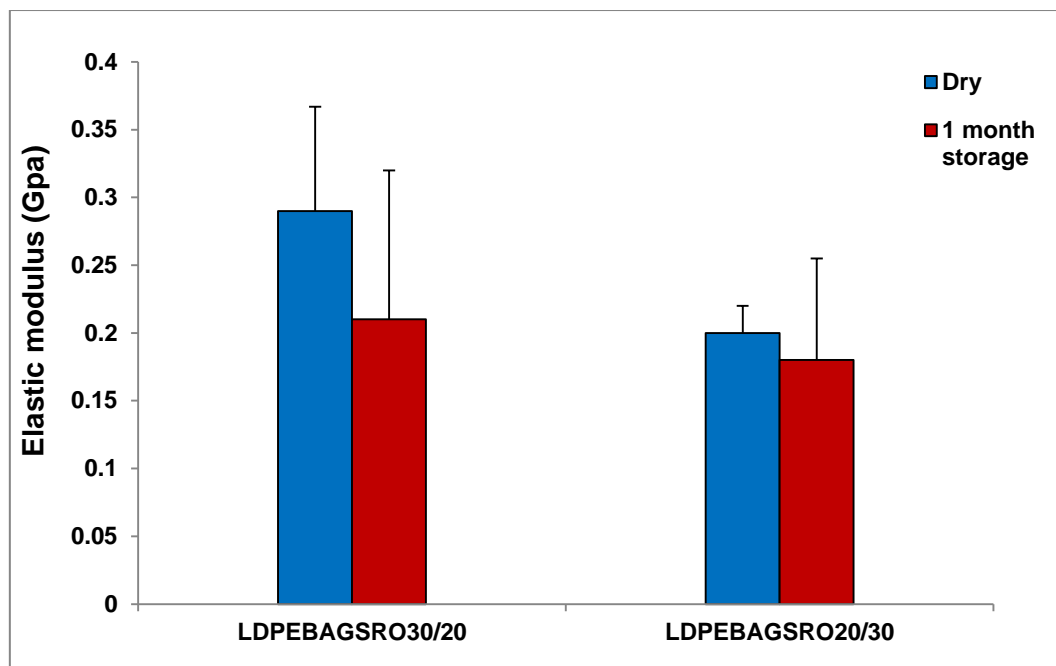


Figure 6.3: Elastic moduli of the polyethylene Bioglass® composites tested both under dry and wet conditions (n=6).

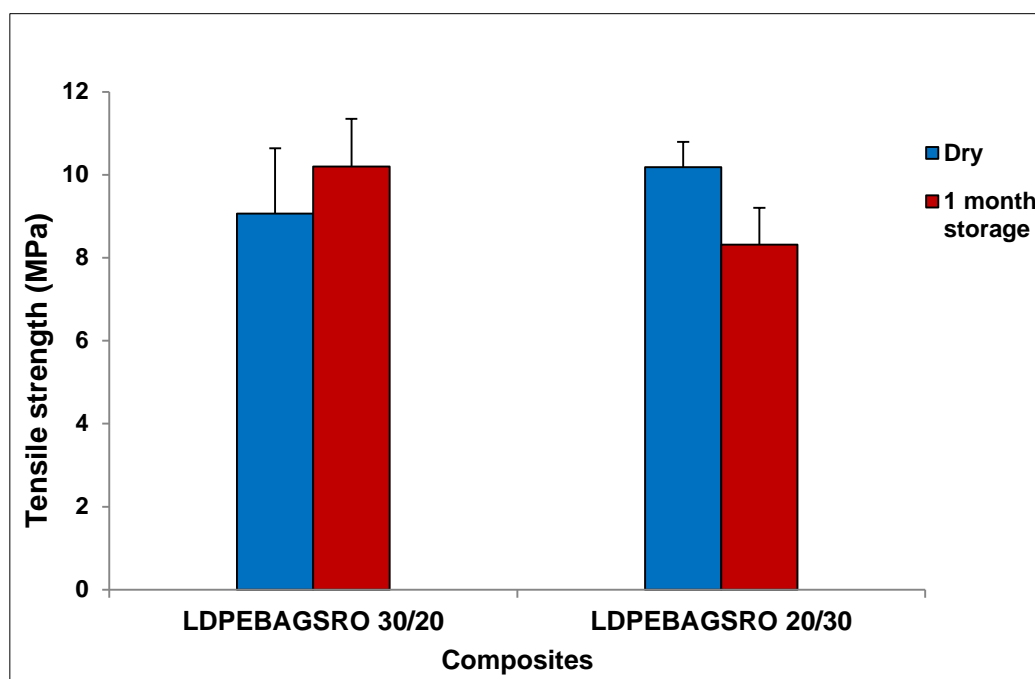


Figure 6.4: Tensile strength of the polyethylene Bioglass® composites tested both under dry and wet conditions (n=6).

Table 6.1: Tensile strength and elastic moduli of the polyethylene-Bioglass[®]/SrO composites and gutta-percha; (n=6).

	Dry		Wet	
	Tensile strength (MPa) Mean±SD	Elastic modulus (GPa) Mean±SD	Tensile strength (MPa) Mean±SD	Elastic modulus (GPa) Mean±SD
LDPEBAGSRO 30/20	9.06 ± 1.57	0.29 ± 0.077	10.2 ± 0.61	0.21 ± 0.11
LDPEBAGSRO 20/30	10.1 ± 1.15	0.20 ± 0.02	8.3 ± 0.88	0.18 ± 0.075
Gutta-percha	7.03 ± 0.8	0.074 ± 0.01	8.04 ± 0.86	0.080 ± 0.01

6.3.3 DSC analysis

The incorporation of Bioglass[®] as particulate fillers into polyethylene matrix showed no significant changes in the melting and crystallisation temperatures of the experimental composites. The thermograms of LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites are shown in, Figure 6.5 & 6.6 and summarized in Table 6.2.

Table 6.2: The melting and crystallization temperatures of the polyethylene-Bioglass[®]/SrO composites; (n=3).

Composite	T _m /°C	T _c /°C
LDPEBAGSRO20/30	111.1	93.1
LDPEBAGSRO30/20	112.6	92.5

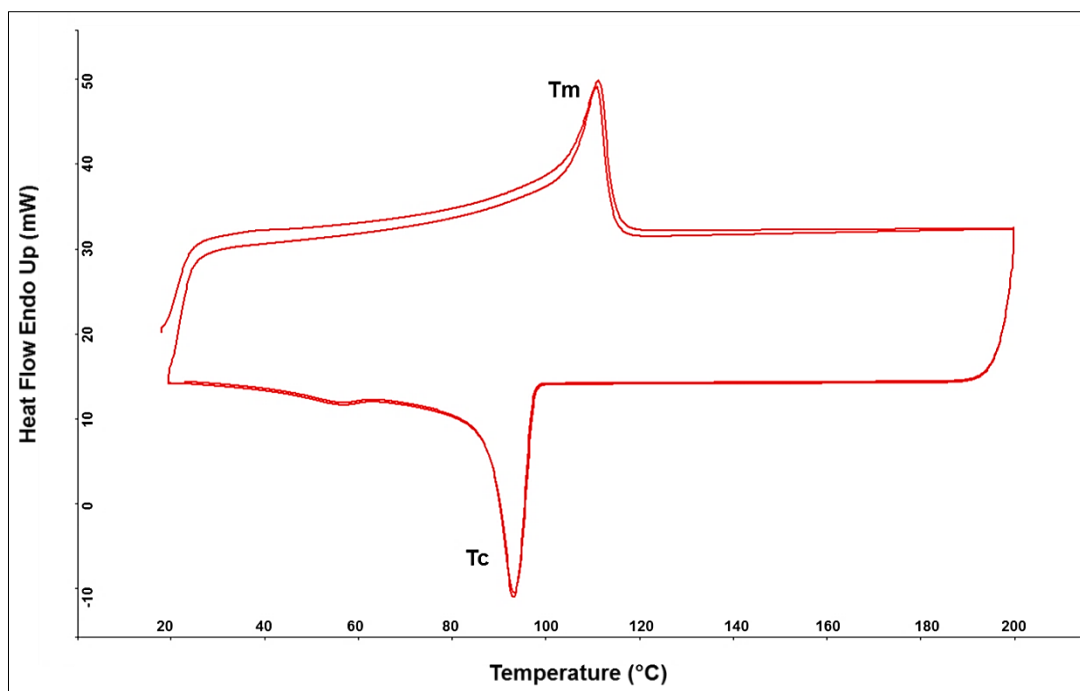


Figure 6.5: DSC thermograms of LDPEBAGSRO 20/30 composite, with the two heating and cooling cycles.

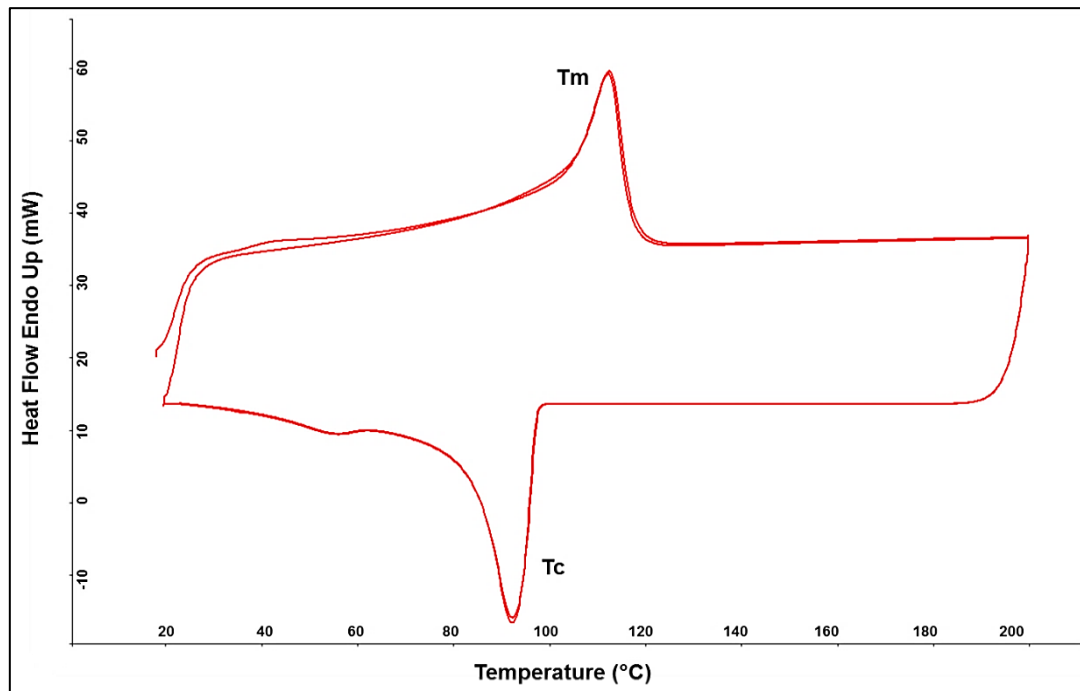


Figure 6.6: DSC thermograms of LDPEBAGSRO 30/20 composite, with the two heating and cooling cycles.

6.3.4 Physical integrity of the polyethylene-Bioglass[®] composites after sterilisation with Gamma irradiation

The integrity of the polyethylene-Bioglass[®] composite in terms of weight, length and thickness was characterised after gamma irradiation using an electronic calliper (Maple electronics). The findings of the test are summarized in Table 6.3, that showed no significant changes in the weight and dimensions of the experimental polyethylene-Bioglass[®] composites.

Table 6.3: Low density polyethylene Bioglass[®] composites before and after gamma radiation; (n=3).

Composite	Wt before radiation (g)	Wt after radiation (g)	Length before radiation (mm)	Length after radiation (mm)	Thickness before radiation (mm)	Thickness after radiation (mm)
LDPEBAG SRO 20	0.0201	0.0201	25.00	25.00	1.10	1.10
LDPEBAG SRO 30	0.0201	0.0201	25.00	25.00	1.10	1.10

6.3.5 X-ray contrast

The degree of radiopacity of the LDPE/BAG 20 & LDPE/BAG 30 composites was assessed using digital radiography and analysed with image (J) software. The mean density of the LDPE/BAG 20 composite condensed in the aluminium washer was 140 which is equivalent to around 4.1 mm Al when compared with steps of aluminium step wedge as shown in Figure 6.7

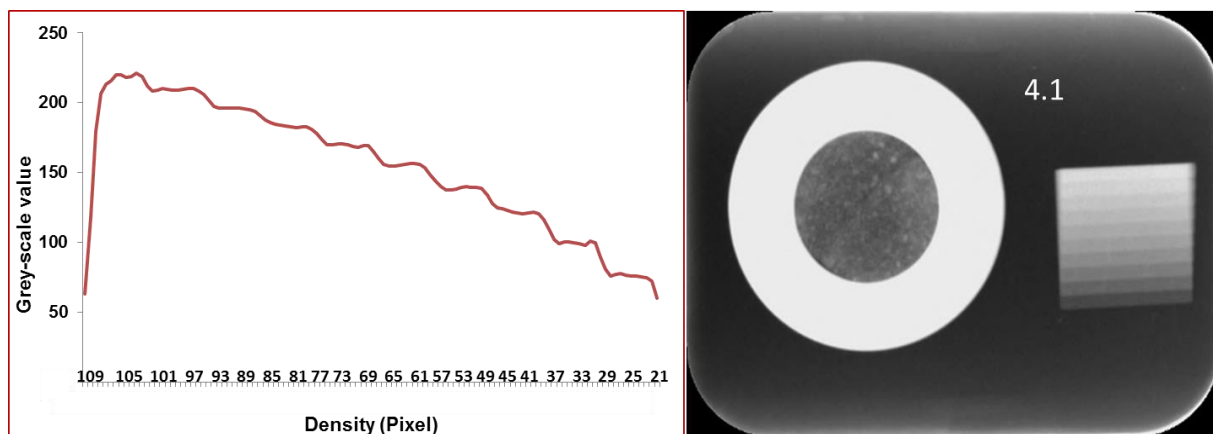


Figure 6.7: Radiograph shows the radiopacity of the LDPE/BAG 30 composite in the washer estimated in relation to the density of the aluminium step wedge using image J software plot profile analysis (n=5).

On the other hand, the LDPE/BAGSrO 20/30 exhibited a radiopacity lower than that of LDPE/BAGSrO 30/20 because the mean density of the LDPE/BAG 20 was obtained at 130 pixel which is equivalent to around 3 mm Al as shown in Figure 6.8.

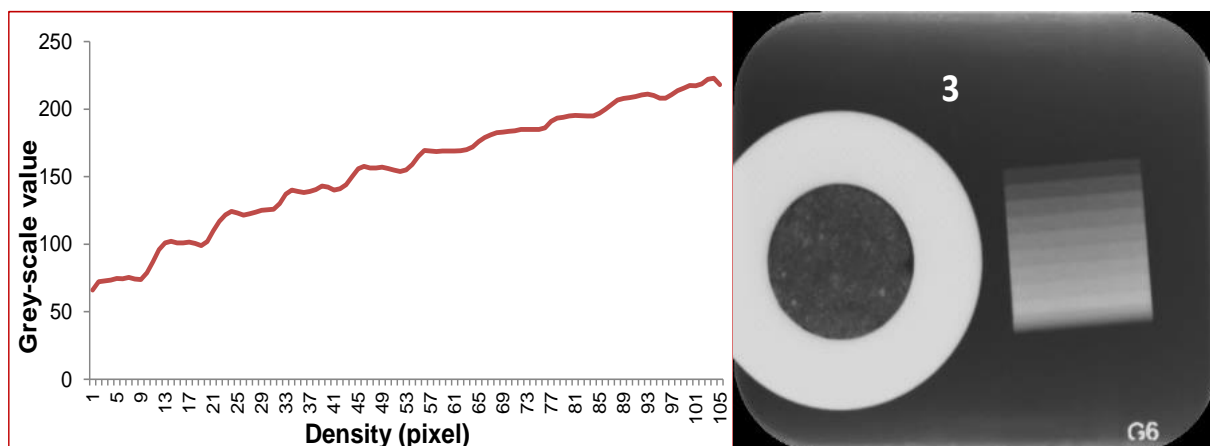


Figure 6.8: Radiograph shows the radiopacity of the LDPE/BAG 20 composite in the washer estimated in relation to the density of the aluminium step wedge using image J software plot profile analysis (n=5).

6.3.6 Biocompatibility

6.3.6.1 MTT assay

LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites exhibited no deleterious effects ($P > 0.05$) on the metabolic activity of cells at 1 and 3 day exposure compared to the negative non-toxic control group as shown in Figure 6.9 & 6.10. The quality and the quantity of the Bioglass[®] filler particles did not show any significant influence on the eluants. Cell exposed to eluants from the test materials showed a non-significant drop in metabolic activity at 24-h & 72-h following 1 day cell exposure as shown in Figure 6.9.

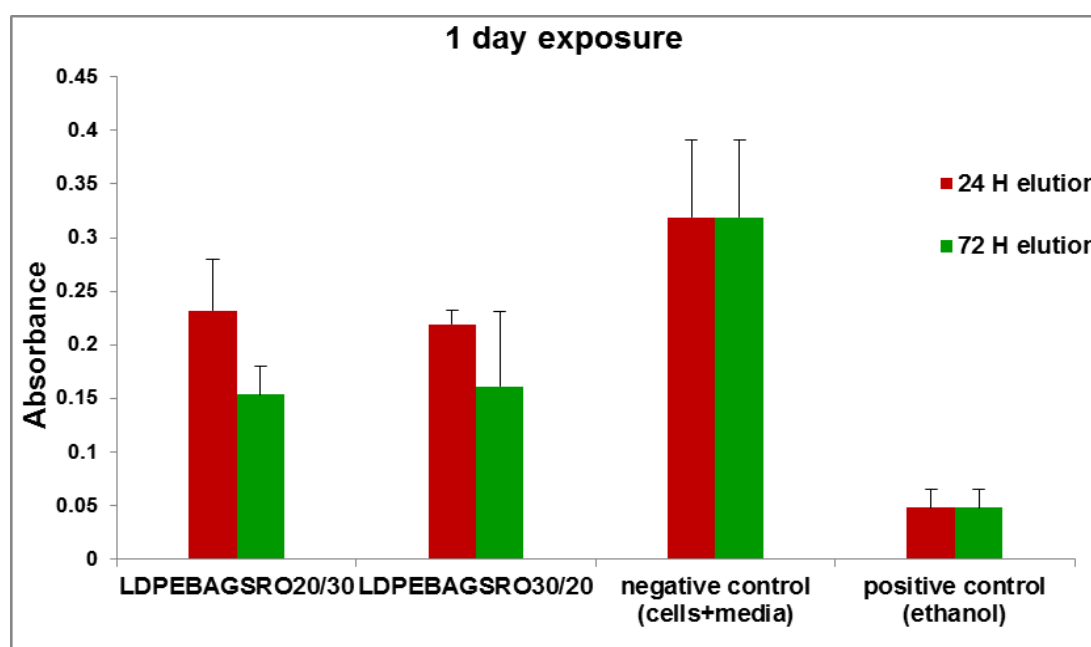


Figure 6.9: Viability of human osteoblast-like cells following 1 day exposure detected at 24 h and 72 h eluated media from composites containing Bioglass[®] expressed as mean and SD absorbance.

Following 3-day cell exposure, there was a significant improvement in the metabolic activity of the cell detected at 24-h and 72-h elution fluids compared with the non-toxic control group, indicating a good biocompatibility particularly at 24-h elution of LDPEBAGSRO 30/20 composite after 3 day exposure as shown in Figure 6.10.

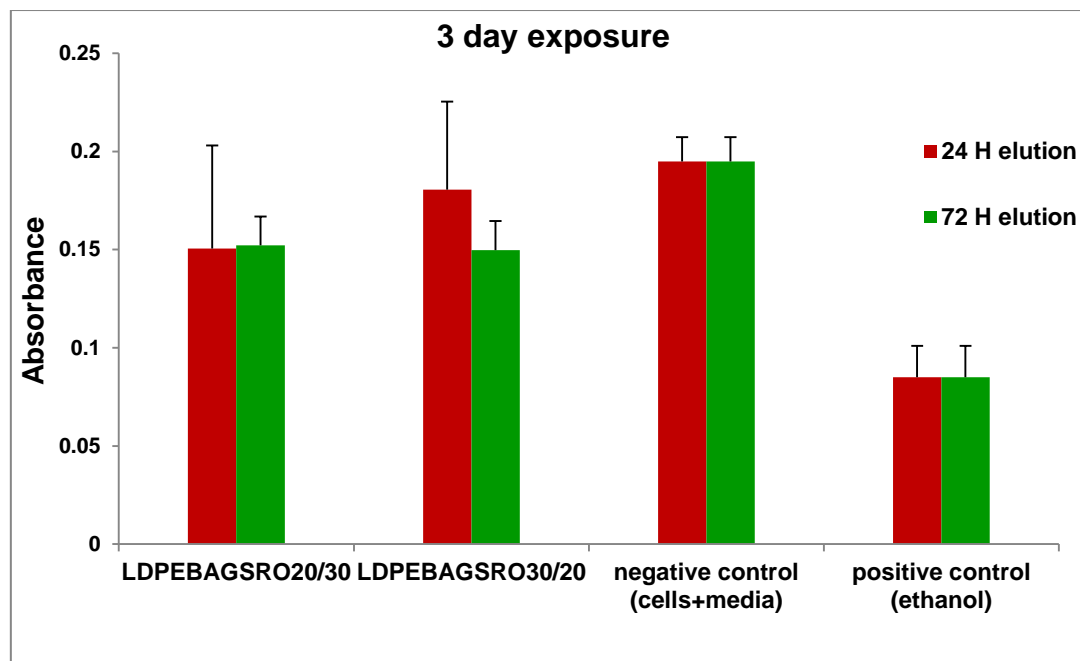


Figure 6.10: Viability of human osteoblast-like cells following 3 day exposure detected at 24 h and 72 h eluted media from the composite containing Bioglass[®] expressed as mean and SD absorbance.

6.3.6.2 alamarBlue assay[™]

The biological response of human osteoblast-like cells on the Bioglass[®] reinforced polyethylene composites for 28 days compared with Thermanox[™] (control) are shown in Figure 6.11. Cell proliferation on the LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites showed no significant difference ($P > 0.05$) in comparison to the non-toxic control Thermanox[™] during 28 days with a slight encouragement in cell proliferation between the 14th and the 21th days in the LDPEBAGSRO 20/30 composite compared to Thermanox[™].

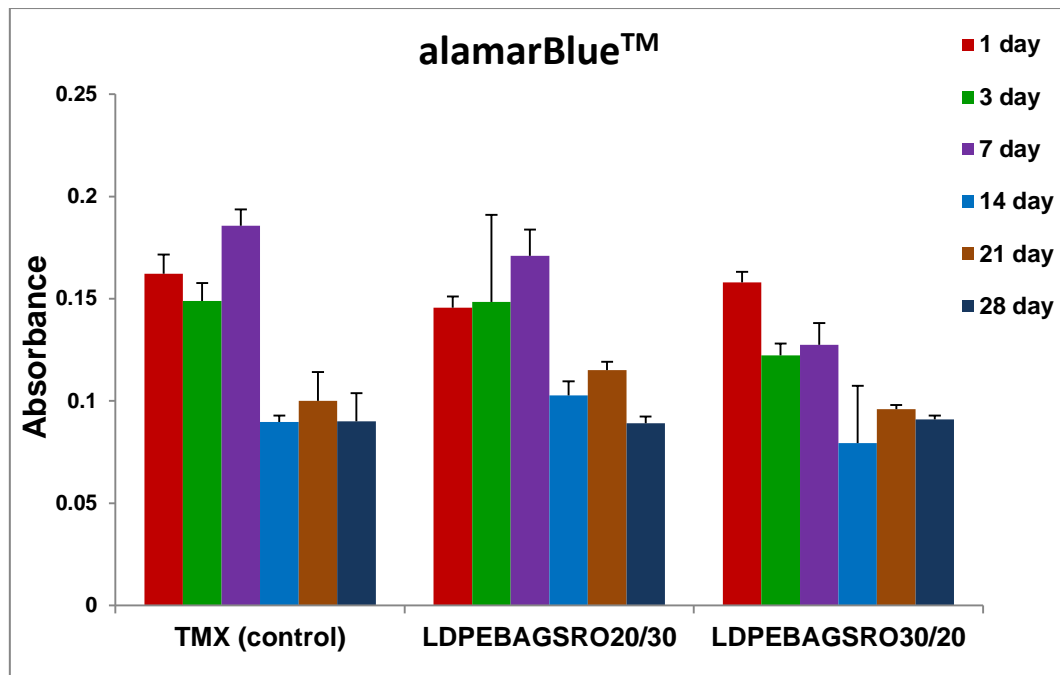


Figure 6.11: Proliferation of human osteoblast-like cells as indicated by alamarBlue™ assay in direct contact with composites containing Bioglass® at 1, 3, 7, 14, 21 and 28 days.

6.3.7 Scanning electron microscopy (SEM)

The dispersion and distribution of Bioglass® fillers in the low density polyethylene matrix before and after immersion in SBF was examined using scanning electron microscopy. For the composites before immersion in SBF, the Bioglass® particles were evenly distributed in low density polyethylene matrix. The amount of Bioglass® on the composite surface increased with content. After 2 days immersion of the composites in the SBF, Bioglass® reacted with the solution, which established sites for apatite nucleation leading to the growth of apatite-like crystals, covering the surface of the composite. The spread of the apatite layer over the low density polyethylene matrix depends on the amount of Bioglass® fillers in the composite. Thus, the hydroxy-carbonate apatite-like layer (HCA) was observed on the external surface of the composites. The micrographs of the SEM are illustrated in Figure 6.12 A, B, C and D.

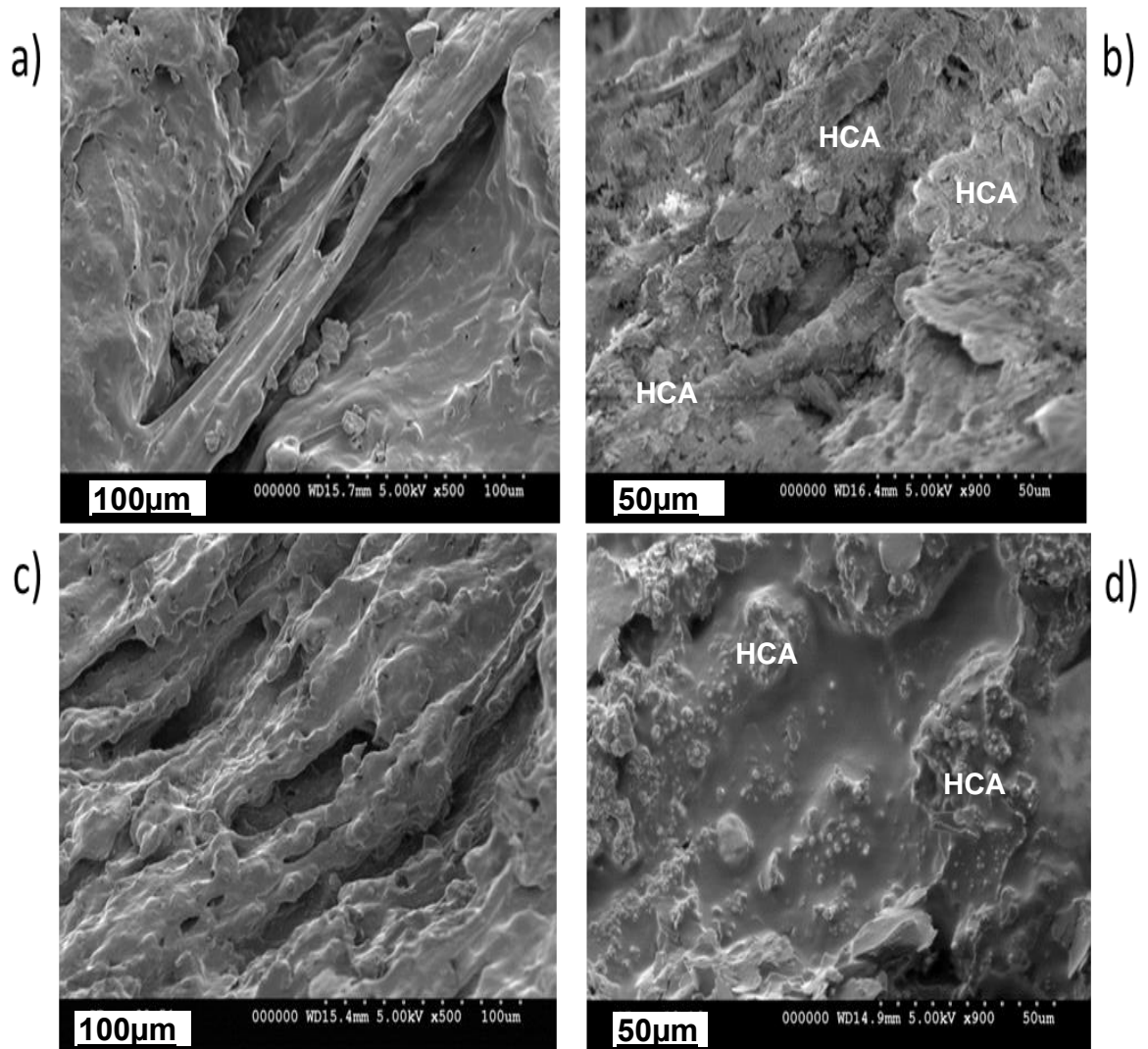


Figure 6.12: SEM micrographs of the polyethylene Bioglass® 20% composite (a) before immersion in SBF, (b) after immersion, and 30 % Bioglass® (c) before immersion, (d) after immersion.

6.3.8 Discussion

The field of endodontics is expanding and one of the recent trends has been the development of bonded root canal obturation materials in an effort to provide a more effective seal. In addition, bioactive materials such as mineral trioxide aggregate (MTA) have extended applications in endodontics and furthermore it is being recognized that materials that are able to reinforce the tooth against fracture are of interest.

Bioglass[®] reinforced low density polyethylene composites as root canal obturation materials are reported, using a melt extrusion process. A single screw extruder with high shear forces was employed in the current study to disperse Bioglass[®] fillers in polyethylene matrix leading to a homogenous distribution of particles in the matrix of the composite. A previous study by Wang *et al* (1998) also showed that a good dispersion of Bioglass[®] was achievable in a high density polyethylene matrix, however a twin screw extruder was required for processing these composites enabling formation of Bioglass[®] reinforced composites for several biomedical applications (Wang *et al*. 1998). The current study was aimed at developing bioactive composites that could be used for endodontic obturation by modifying the adaptability of material to conform to the complexity of root canal wall. 45S5 Bioglass[®] was incorporated into polyethylene matrix to achieve the bioactivity which may be needed to improve the sealing ability of the material within root canal system. The particle size of Bioglass[®] was predetermined in a pilot study; the amount of Bioglass[®] filler was maintained at 20% and 30% by weight with the addition of 20% and 30% by weight of SrO to impart the radiopacity to the material. The polyethylene matrix composition was maintained at 50% by weight in both the experimental composites. The processing parameters for extrusion were set such that the first zone of the

extruder barrel ensured mixing with the following zones forming a smooth melt mix prior to the fibre being extruded from the die.

The bioactivity of the composite was determined as described by Kokubo *et al* (2003) by allowing the interacting of the composites with simulated body fluid at physiological temperature (Kokubo *et al.* 2003). After a series of surface reactions, a hydroxy-carbonate apatite-like layer was observed on the surface of the material. Formation of a biological apatite-like layer on the surface of Bioglass[®] reinforced low density polyethylene composites was characterised *in vitro* using FTIR. Ionic reactions of Bioglass[®] with the SBF occur after immersion of the composite in the fluid leading to the formation of silanol, through which the apatite layer grows and deposits on the material surface. It is apparent from the FTIR spectra that the presence of phosphate and carbonate absorption bands confirmed the formation of an apatite-like layer which is hydroxy-carbonate apatite on the surface of the material. These findings are consistent with previous studies (Vallet-Regi *et al.* 1999, Juhasz *et al.* 2003), describing the formation of an apatite-like layer on the polyethylene matrices. Thus, the hydroxy-carbonate apatite layer was established on composite surfaces indicating the *in vitro* bioactivity of the composite. Such an interaction may be particularly advantageous in the apical region of the root canal space where the Bioglass[®] composites come in contact with the fluid coming from the periapical tissues. Another beneficial response of Bioglass[®] is their high alkalinity, which may elicit an antibacterial effect and thus decrease bacterial colonisation within the root canal space (Allan *et al.* 2001).

The tensile strength tests of Bioglass[®] reinforced polyethylene composites were undertaken in dry and wet conditions (1 month storage in SBF). The tensile strength and elastic modulus data showed that the polyethylene-Bioglass[®]

composites exhibited stiffness, approaching that of dentine, which is significantly higher than that of gutta-percha values when tested under dry conditions and after 1 month storage in simulated body fluid (SBF). The tensile strength and elastic modulus of gutta-percha have been reported in the literature to be around 7 MPa and 77 MPa, respectively (Williams *et al.* 2006). It is believed that the higher modulus of root canal obturation materials could help reinforce the endodontically treated teeth and prevent root fracture (Grande *et al.* 2007, Bishop *et al.* 2008). The inclusion of Bioglass[®] into low density polyethylene produced a composite with higher modulus in comparison to the unfilled low density polyethylene. The elastic modulus of Bioglass[®] reinforced polyethylene composite significantly increased with an increase in the weight fraction of Bioglass[®] particles ($P < 0.05$) in the composite, whereas the tensile strength decreased. These findings are supported by previous study (Wang *et al.* 1998), wherein 10%, 20% and 40% of Bioglass[®] by volume were incorporated into high density polyethylene and they found a drop in the tensile strength and an increase in the elastic modulus with an increase in the Bioglass[®] volume. Furthermore, the modulus values of dry composites with 20% and 30% of Bioglass[®] by weight were found to be superior to those of the same composites tested under wet conditions. This may be accounted by the ionic release from Bioglass[®] filler when it comes in contact with the solution after one month storage, resulting in a decrease in the content of Bioglass[®] in the composite. It is apparent from the results of the tensile test that the stiffness of polyethylene/Bioglass[®] composites are significantly ($P < 0.05$) lower than those of polyethylene/hydroxyapatite composites as Bioglass[®] is a glass, which has a smoother surface, giving rise to a weaker coupling, whereas hydroxyapatite is a ceramic that has a rough, micro-mechanically retentive surface.

The melting temperatures of Bioglass[®] reinforced polyethylene composites was characterised using DSC. The melting temperatures of Bioglass reinforced polyethylene composites were in the range of 111-112°C, indicating that this material would be easy to remove using heat application with no complication in cases of retreatment. The incorporation of Bioglass[®] fillers into low density polyethylene matrix did not affect the melting temperature of the composite.

A primary human osteoblast-like cell culture model was used to test MTT and alamarBlue™ assays to characterise the biological response of the Bioglass[®] reinforced polyethylene composites. The MTT assay showed no deleterious or cytotoxic effects on the viability and metabolic functions of osteoblast-like cells suggesting a good biological response with vital tissue with no leachable toxic ions released. The initial lowering of MTT activity at 1 day exposure expressed on cell can be attributed to the increase in the local pH of a Bioglass[®] reinforced polyethylene composite due to the interaction of the medium with Bioglass[®] from the composite, which may cause the release of sodium ions present in the glass. At day 3 of the MTT assay, a significant increase in the cellular metabolic activity was observed, suggesting that Bioglass[®] in the composite has a stimulatory effect on the cellular activity through establishing favourable sites for cell adhesion and growth. This may be attributed to the release of silicon which is essential for bone formation. Huang *et al* (1997) have shown that composites of Bioglass[®] reinforced high density polyethylene have an excellent biological response as a potential implant material and significantly increase the metabolic activity of human osteoblast like cells. Furthermore the alamarBlue™ test showed an enhanced proliferation of human osteoblast cells after 14 days of cell culture. This finding may be related to the release of sodium, calcium, phosphorus and silicon ions from 45S5 Bioglass[®] producing cellular adhesion and cellular

proliferation (Hench & Andersson 1993). Furthermore, the inclusion of SrO as radiopacifier into the composites did not change the bioactivity of the material.

Radiopacity is one of the essential requirements for intra-oral dental materials. Root canal obturation materials need to be radiopaque enough to allow for discrimination between the materials and the adjacent anatomical structures as well as to assess the quality of the endodontic obturation. Thus, the radiopacity of the LDPE/BAG 20 and LDPE/BAG 30 composites was assessed with digital radiography and storage-phosphor system. In accordance with the standards established by (ANSI/ADA 2000) Specification No. 57, root canal obturation materials should possess a radiopacity not less than that equivalent to 3 mm of aluminium. The composites of Bioglass[®] reinforced polyethylene showed a radiopacity greater than 3 mm of aluminium equivalent; the radiopacity of LDPE/BAG 20 was superior to that of LDPE/BAG 30 due to higher amount of SrO present in LDPE/BAG 20 composite.

The overall properties of the PE-Bioglass[®] composites clearly indicate their suitability as potential obturating materials; however, further investigations are necessary to assess their sealing ability and antibacterial activity

6.3.9 Conclusion

Bioglass[®] reinforced low density polyethylene with SrO composites are promising obturation material for endodontic treatment. They exhibit superior stiffness and the low melting temperature is expected to allow facile removal in case retreatment is required. The composites also exhibit good biocompatibility and bioactivity, which are beneficial properties. An enhanced proliferation of human osteoblast cells was also observed.

Chapter 7 General Summary & future work

The success of a root canal treatment depends on three main factors, namely, thorough canal debridement, effective disinfection and obturation of the root canal space. One of the most critical components in the success of root canal treatment has been the obturation of the root canal space and the maintenance of a good seal, which has led to a number of new obturating materials with some being launched commercially in the last decade (Li *et al.* 2014b). The development of new materials or techniques for root canal obturation must be aimed at entombing residual bacteria and preventing recontamination via achieving a thorough seal of the root canal space and improving the sealability. Another important consideration is the easy removal of the obturating material when endodontic retreatment is required due to the failure of the primary procedure. As mentioned earlier in the literature, gutta-percha is the most commonly employed obturating material, which is used in conjunction with a sealer. Although the properties are acceptable it has a number of shortcomings such as lack of rigidity and easy displacement under pressure. Resilon™ is another more recent obturating material that is based on polycaprolactone, a biodegradable polymer, which has been shown to elicit a more favourable periapical response than gutta-percha obturations when tested *in vivo* in dogs (Shipper *et al.* 2005), however some studies indicate that there were no differences (Leonardo *et al.* 2007, Brasil *et al.* 2010). Although Resilon™ has been reported to provide a good sealing ability with existing sealants (Duggan *et al.* 2009), it cannot yet be considered an evidence-based replacement to gutta-percha and prospective clinical studies comparing Resilon™ to currently available materials are needed (Shanahan & Duncan 2011).

Endodontic treatment compromises the strength of the tooth. The risk of fracture of roots weakened has led some clinicians to reinforce the coronal part of the tooth by composite cements and obturations and by attempting to use integrated systems such as a resin core with adhesive obturations. Although there is some evidence of the benefit of such systems, clinical data is limited. It is also thought that the success of the root canal treatments is dependent on the reinforcing effect of root canal obturating material (stiffness), which is expected to help prevent root fracture and thereby increase the survival of root canal treated teeth. However, the currently available adhesive root canal obturation materials are unable to sufficiently strengthen roots (Ungor *et al.* 2006, Grande *et al.* 2007, Ribeiro *et al.* 2008). It is acknowledged that in order to strengthen root filled teeth a material must bond to root canal walls however, if the stiffness of the material is not close enough to that of dentine, the adhesive procedures alone are not sufficient to reinforce the root (Grande *et al.* 2007). Williams *et al.* (2006) concluded that the stiffness of Resilon™ and gutta-percha are not sufficient to strengthen roots after root canal therapy as the elastic moduli of gutta-percha and Resilon™ are too low (0.077 GPa and 0.129 GPa, respectively) in comparison to that of dentine (14-17 GPa). Thus, the overall aim of this *in vitro* study was to develop, characterise and assess newer materials for endodontic applications.

Carrier-based obturation systems are effective in obturating the root canal space in three dimensions creating good adaptation of the material to the root canal wall. They provide a convenient method of delivering thermally softened gutta-percha into the root canal systems with some degree of control (Kytridou *et al.* 1999, Li *et al.* 2014a). One challenging aspect of the currently available carrier-based obturation systems (i.e. Thermafil®) is the difficulty associated with their removal during retreatment cases (Ibarrola *et al.* 1993). Polysulfone Thermafil®

obturator are not soluble in commonly used endodontic solvents, also their melting temperature is extremely high, around 350°C, which makes their retrievability difficult. The current study thus explored specifically designed materials shaped as carriers and coated with a thin layer of gutta-percha to function as a carrier-based root canal obturation system. Potential composite carriers with better physical (i.e. low melting temperature) and mechanical characteristics (flexibility) were developed in the present work and compared to the current carrier-based obturation systems. The composition of the newly designed composite carriers include hydroxyapatite reinforced polyethylene composites or polylactic acid/polyhydroxybutrate-hydroxyapatite composites. The newly designed carriers made of polyethylene-based composites are easily removed due to their low melting temperature (around 112°C) whereas polyester-based composites can be removed effortlessly due to their solubility in calcium hydroxide paste.

7.1 Polylactide/polyhydroxybutrate-hydroxyapatite composite as core-carriers for carrier-based root canal obturation

In this study, polyhydroxybutrate (PHB) and polylactic acid (PLA) were selected to form the matrix of the composite with hydroxyapatite as the filler. The selection was based on thermoplasticity, excellent biocompatibility, processability (Joseph *et al.* 2002), and the ability to tune their properties by changing the composition of the composites. The application of polyesters was based on the fact that their removal could be facilitated by using alkaline mediated hydrolysis (Khan *et al.* 2004) in cases of retreatment and post space preparation. Although these groups of polymers are biodegradable, PHB exhibits an extremely slow rate of degradation and furthermore both PLA and PHB predominantly degrade via a hydrolytic mechanism thus; these polymers do not degrade or exhibit minimal

changes in properties in the absence of water. The purpose for developing these composites was to form core-carriers for endodontic obturation. The polylactic acid and polyhydroxybutrate/hydroxyapatite ratios were optimised to meet the clinical handling considerations. The present study showed disintegration of Polylactide/polyhydroxybutrate-hydroxyapatite composites when placed in non-setting calcium hydroxide paste. This is an expected finding since PLA and PHB are known to undergo hydrolytic degradation (Zhang & Thomas 2011) which is accelerated in alkaline medium. A similar finding was reported when complete degradation of a PLA/HA composite was observed within 48 hours of interaction with calcium hydroxide pastes (Nooshin 2010). Cytocompatibility of the obturating material is an absolute requirement that was confirmed via *in vitro* cellular studies using alveolar human osteoblast like cells. No deleterious effect was observed on the cell metabolic and cell proliferation activity as shown by MTT and alamarBue™ assay tests.

However, the tensile test results showed that PLA/PHB-HA composites were brittle and rigid; this may limit their use for obturation of the root canal space.

7.2 Polyethylene hydroxyapatite composites as both an obturation core material and core-carriers for endodontic treatment

Composites comprising of polyethylene matrix with different filler concentrations of silanated and non-silanated hydroxyapatite with strontium oxide as a radiopacifying agent were developed, for use as both obturation core material and core carrier. The rationale for the use of polyethylene-hydroxyapatite composites for endodontic applications was based on the fact that composites of high density polyethylene and hydroxyapatite exhibit excellent biocompatibility with good mechanical properties. In addition hydroxyapatite reinforced high

density polyethylene composites were used successfully in biomedical applications as implant materials in the orbit and middle ear and in the skull (Bonfield *et al.* 1981, Abu-Hammad *et al.* 2000, Zhang *et al.* 2007). Polyethylene has generated a great deal of interest in the last few decades for biomedical and dental applications because of its excellent biocompatibility, thermoplasticity, processability, and high flexibility; thus polyethylene was used in this study in conjunction with hydroxyapatite (HA) fillers. Hence to adapt this technology for developing a root canal obturation material, a pilot study was undertaken at the beginning of this research project, to allow processing and yield materials, compliant with the clinical handling requirements in endodontics. The amount of hydroxyapatite filler is a significant parameter that can help in tailoring composites with certain degree of flexibility considering that clinical handling is the utmost priority.

In the design of the low density polyethylene hydroxyapatite composites to function as carriers, hydroxyapatite particles were added to low density polyethylene in two concentrations of 20% and 40% to improve and optimise the mechanical properties of the composite material and also impart the radiopacity. Additionally, 10% SrO, a heavy metal oxide was also included to enhance the radio-contrast as required for an endodontic material to enable radiographic evaluation post treatment. The composites with 20% HA showed promising results to be used as carrier in comparison to the composites with 40% HA as they exhibited excellent flexibility and homogeneity in their structure. The results indicated that high concentration of the filler compromised the handling properties required for a carrier grade material; hence a lower concentration of the filler yielded the requisite characteristics.

For use as obturation materials, two sets of composite fibres were produced by incorporating 20 and 30% by weight of HA particles into the low density polyethylene resin matrix with 30% SrO and 20% SrO, respectively. The study involving the formation of the carrier material, clearly indicated that 40% by weight of hydroxyapatite resulted in the production of fibres that were rigid and thus the maximum amount of HA filler for the obturation material was restricted to 30% by weight. The quantity of SrO radiopacifier in these composites was increased with the aim of achieving radiopacity required for any root obturation material, meanwhile, this increase of SrO produced no significant effect on the physical and thermo-mechanical properties of the composites. The enhancement of the mechanical properties of polyethylene with the addition of hydroxyapatite has also been reported by several investigators wherein addition of HA particles to high density polyethylene improved the material rigidity to function as bone analogues and implant materials (Bonfield 1988, Tanner 2010).

The PE-HA composites were also processed with HA particles treated with A-174, a silane coupling agent, to enhance the bond strength between the filler and polymer at their interface and achieve a durable composite material (Deb *et al.* 1996, Wang & Bonfield 2001). Owing to hydrophobicity of polyethylene polymer and hydrophilicity of HA, debonding of the composite at the filler matrix interface was expected (Friedrich & Karsch 1983, Bonfield *et al.* 1998). Hence the ability of the silane coupling agent to bond chemically on the HA surface was essential in promoting the interfacial adhesion between the matrix and filler. Overall the newly developed polyethylene hydroxyapatite composites offered relevant benefits as they exhibited a good elastic modulus and tensile strength in comparison to gutta-percha and Resilon™. *In vitro* cell culture studies represented by MTT and alamarBlue™ assays showed an encouraging proliferation of the human

osteoblast cells. The reason for enhanced proliferation of the HOB cells could be related to the presence of hydroxyapatite crystals in the composites which provide favourable sites for cellular adhesion and attachment, and subsequently matrix deposition. Another explanation of the enhanced cellular behaviour may be related to the surface irregularity of the hydroxyapatite reinforced polyethylene composites. In addition to the tailor-made stiffness and excellent biocompatibility, hydroxyapatite reinforced polyethylene composites can be heat-sterilised offering a further advantage that is lower melting point precludes such action.

SrO was also incorporated into composites to impart the radiopacity required for any root canal obturation material. A speculated additional advantage of the inclusion of SrO is that it may exert an antibacterial activity, this is supported by a previous study (Guida *et al.* 2003), wherein they reported the importance of use of strontium containing materials to kill the residual bacteria preventing recontamination of the dental treatment, however this needs to be investigated.

7.3 Polyethylene Bioglass[®] composite as an obturation core material for endodontic treatment

There is recognition of merit for using not only biocompatible but bioactive materials in endodontic treatment. For example MTA is a bioactive and biocompatible material MTA, which exhibits excellent biological properties both *in vivo* and *in vitro* that have been also shown to enhance proliferation of periodontal ligament fibroblasts; thus MTA based root canal sealers have been proposed. Another interesting study reported that bioactivity in endodontic obturation could improve the sealing ability particularly in the apical region of the root canal due to the presence of the moisture that comes from both the periapical tissue and dentinal tubules (Enkel *et al.* 2008). Composites of Bioglass[®] filler reinforced low density polyethylene were developed with the aim

of producing unique biological response between the material itself and the adjacent tissue forming a bioactive bond via producing a hydroxy-carbonate apatite-like layer (HCA) when immersed in physiological fluid. The high alkalinity of the glass phase of Bioglass[®] composites has been reported to impart antibacterial effects to the polymer composites (Allan *et al.* 2001, Koller *et al.* 2008). The possibility of controlling the rate of bonding to tissue is an important aspect that discriminates the use of Bioglass[®] from hydroxyapatite (Hench *et al.* 1971, Hench 1998). 45S5 Bioglass[®] was chosen over other Bioglass[®] because it has the ability to bind to both soft and hard tissues whilst other Bioglass[®] bind only to bone (Hench 1998). The biocompatibility studies were carried out using *in vitro* cell culture tests, MTT and alamarBlue™ that showed no deleterious effect on the metabolic activity of human osteoblast cells which exposed to the Bioglass[®] reinforced polyethylene composites indicating good cellular response of the material. Furthermore, a stimulatory effect on cellular metabolic activity was observed due to the presence of Bioglass[®] in the composite. This effect may be related to the release of ions from Bioglass[®] particularly through surface interaction, which may play a vital role on bone formation (Hench & Andersson 1993). The positive results of the Bioglass[®] reinforced polyethylene composites on human osteoblast-like cells are in accordance with a previous study (Huang *et al.* 1997).

It is interesting to note that the elastic modulus and tensile strength of hydroxyapatite reinforced polyethylene composites were significantly higher than those of Bioglass[®] reinforced polyethylene composites.

Polyethylene Bioglass[®] composite offers significant benefits over polyethylene hydroxyapatite composite due to bioactivity and high alkalinity.

7.4 Conclusion

The following conclusions can be drawn:

1. Promising new root canal obturation materials based on polyethylene silanated hydroxyapatite or Bioglass[®] with SrO composites were designed for endodontic obturation. The composites of LDPEHA with different concentrations of HA and SrO were found to be suitable for root canal obturation by virtue of their mechanical properties and radiopacity. They exhibited superior elastic modulus and tensile strength in comparison to gutta-percha. Furthermore, the material properties are expected to reinforce the endodontically treated root, with the additional advantages being the ability to heat-sterilise, easy retrieveability and excellent biocompatibility.
2. The current study established that the specifically-designed composite of 70% polyethylene and 20% silanated hydroxyapatite with 10% SrO had suitable properties to find application in endodontic obturation as carriers for carrier-based root canal obturation. The composite demonstrated better physical properties (low melting temperature) and excellent flexibility in comparison to other current carrier systems with desirable radiopacity. Based on the melting temperatures of the composites designed for carrier-based obturation, the currently available Thermafil[®] Prep plus oven was modified and deemed to be compatible with the composite fibres fabricated in the current work.
3. The suitability of polyhydroxybutrate/polylactic acid with hydroxyapatite composites designed to function as carriers for carrier-based obturation system was limited because of their brittleness and further investigations are warranted.
4. Based on the cellular response, the composites containing hydroxyapatite or Bioglass[®] fabricated in the present study were found to be biocompatible and the results showed a significant increase in the metabolic activity of the cells,

suggesting that hydroxyapatite or Bioglass[®] had a stimulatory effect on human osteoblast-like cells *in vitro*.

7.5 Suggestions for future work

The present study provides evidence that low density polyethylene composites fabricated either with hydroxyapatite or Bioglass[®] with SrO composites have potential as a root canal obturation material. Further research is needed to develop root canal sealant materials and to characterise their bondability to the polyethylene hydroxyapatite composites.

The present research has also paved way for further studies to fabricate fibre posts for restoration of endodontically treated teeth by modulating both the density of the polyethylene matrix and the amount of hydroxyapatite filler.

The possible antimicrobial effects of the polyethylene Bioglass[®] root obturation materials developed in the present study also need to be assessed, this will be a major advancement in this clinical procedure.

Further improvement in the properties of the composites can be achieved by using nanotechnology innovation via incorporation of antibacterial nano-hydroxyapatite or nano-Bioglass[®] fillers into the polyethylene composites.

Improvement in the brittleness of polyester-based composites fabricated in the current work can be accomplished by incorporating lactide isomers (D-lactide and L-lactide) into PLA resin matrix to obtain (PDLLA), thereby achieving a significant decrease in crystallinity and crystallization rate. Using polydioxanone resin matrix as a root canal obturation could also be considered. Once the mechanical and antibacterial properties of the potential obturating materials are fully assessed their clinical assessment in a randomised clinical trial will be undertaken.

References

- Abu-Hammad OA, Harrison A, Williams D (2000) The effect of a hydroxyapatite-reinforced polyethylene stress distributor in a dental implant on compressive stress levels in surrounding bone. *Int J Oral Maxillofac Implants* **15**(4), 559-64.
- Alani A, Knowles JC, Chrzanowski W, Ng Y-L, Gulabivala K (2009) Ion release characteristics, precipitate formation and sealing ability of a phosphate glass–polycaprolactone-based composite for use as a root canal obturation material. *Dental Materials* **25**(3), 400-10.
- Allan I, Newman H, Wilson M (2001) Antibacterial activity of particulate bioglass® against supra- and subgingival bacteria. *Biomaterials* **22**(12), 1683-7.
- American Association of Endodontists (1994) Appropriateness of care and quality assurance guidelines. Chicago: The association.
- Andreasen JO, Farik B, Munksgaard EC (2002) Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. *Dent Traumatol* **18**(3), 134-7.
- ANSI/ADA 2000 Specification number 57 endodontic sealing material. Chicago, USA: ADA Publishing.
- Aptekar A, Ginnan K (2006) Comparative analysis of microleakage and seal for two obturation materials: Resilon/epiphany and gutta-percha. *J Can Dent Assoc* **72**(3), 245.
- Arcos D, Izquierdo-Barba I, Vallet-Regi M (2009) Promising trends of bioceramics in the biomaterials field. *J Mater Sci Mater Med* **20**(2), 447-55.
- Arksornnukit M, Takahashi H, Nishiyama N, Pavasant P (2004) Effects of heat and pH in silanation process on flexural properties and hydrolytic durabilities of composite resin after hot water storage. *Dent Mater J* **23**(2), 175-9.
- Arola D, Replogel RK (2005) Effects of aging on the mechanical behavior of human dentin. *Biomaterials* **26**(18), 4051-61.
- Asgary S, Parirokh M, Eghbal MJ, Brink F (2005) Chemical differences between white and gray mineral trioxide aggregate. *J Endod* **31**(2), 101-3.
- Ashammakhi N, Rokkanen P (1997) Absorbable polyglycolide devices in trauma and bone surgery. *Biomaterials* **18**(1), 3-9.
- ASTM International (2002) Standard test methods for vulcanized rubber and thermoplastic elastomers–tension. West Conshohocken, PA: ASTM International, ASTM D412-98a(2002)e1.
- Avérous L (2008) Polylactic acid : Synthesis , properties and applications. M. Naceur belgacem & a. Gandini, eds. *Synthesis*, 2006-8.

- Bae W-J, Min K-S, Kim J-J, Kim J-J, Kim H-W, Kim E-C (2012) Odontogenic responses of human dental pulp cells to collagen/nanobioactive glass nanocomposites. *Dental Materials* **28**(12), 1271-9.
- Bakhtiar H, Heidari N, Mehrvarzfar P et al. (2012) In vitro comparative study of the microbial leakage of one-step, thermafil and lateral condensation techniques. *J Contemp Dent Pract* **13**(1), 27-30.
- Barkhordar RA, Nguyen NT (1985) Paresthesia of the mental nerve after overextension with ah26 and gutta-percha: Report of case. *J Am Dent Assoc* **110**(2), 202-3.
- Barletta FB, de Sousa Reis M, Wagner M, Borges JC, Dall'Agnol C (2008) Computed tomography assessment of three techniques for removal of filling material. *Aust Endod J* **34**(3), 101-5.
- Barletta FB, Rahde Nde M, Limongi O, Moura AA, Zanesco C, Mazocatto G (2007) In vitro comparative analysis of 2 mechanical techniques for removing gutta-percha during retreatment. *J Can Dent Assoc* **73**(1), 65.
- Barrieshi-Nusair KM, Qudeimat MA (2006) A prospective clinical study of mineral trioxide aggregate for partial pulpotomy in cariously exposed permanent teeth. *J Endod* **32**(8), 731-5.
- Becker TA, Donnelly JC (1997) Thermafil obturation: A literature review. *Gen Dent* **45**(1), 46-55; quiz 9-60.
- Ben-Nissan B, Choi AH (2006) Sol-gel production of bioactive nanocoatings for medical applications. Part 1: An introduction. *Nanomedicine* **1**(3), 311-9.
- Bertacci A, Baroni C, Breschi L, Venturi M, Prati C (2007) The influence of smear layer in lateral channels filling. *Clin Oral Investig* **11**(4), 353-9.
- Beyer-Olsen EM, Ørstavik D (1981) Radiopacity of root canal sealers. *Oral Surg Oral Med Oral Pathol* **51**(3), 320-8.
- Bishop D, Griggs J, He J (2008) Effect of dynamic loading on the integrity of the interface between root canal and obturation materials. *J Endod* **34**(4), 470-3.
- Black J (1981) Biological performance of materials. New York: Marcel Dekker.
- Blümm E, Owen A (1995) Miscibility, crystallization and melting of poly(3-hydroxybutyrate)/ poly(l-lactide) blends. *Polymer* **36**(21), 4077-81.
- Bogen G, Kuttler S (2009) Mineral trioxide aggregate obturation: A review and case series. *J Endod* **35**(6), 777-90.
- Bonfield W (1988) Composites for bone replacement. *Journal of Biomedical Engineering* **10**, 522-6.
- Bonfield W, Doyle C, Tanner K (1986) "In vivo evaluation of hydroxyapatite reinforced polyethylene composites". In: Christel P, Meunier A, Lee A, eds. *Biological and Biomedical Performance of Biomaterials*, pp. 153-9. Amsterdam: Elsevier.

- Bonfield W, Gryn timer MD, Tully AE, Bowman J, Abram J (1981) Hydroxyapatite reinforced polyethylene--a mechanically compatible implant material for bone replacement. *Biomaterials* **2**(3), 185-6.
- Bonfield W, Tanner K (1997) Hydroxyapatite composite biomaterials—evolution and applications. *Mater World*(5), 18–20.
- Bonfield W, Wang M, Tanner K (1998) Interfaces in analogue biomaterials. *Acta Materialia* **46**(7), 2509-18.
- Boretos JW, Pierce WS (1967) Segmented polyurethane: A new elastomer for biomedical applications. *Science* **158**(3807), 1481-2.
- Bortoluzzi EA, Guerreiro-Tanomaru JM, Tanomaru-Filho M, Duarte MA (2009) Radiographic effect of different radiopacifiers on a potential retrograde filling material. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **108**(4), 628-32.
- Bose S, Banerjee A, Dasgupta S, Bandyopadhyay A (2009) Synthesis, processing, mechanical, and biological property characterization of hydroxyapatite whisker-reinforced hydroxyapatite composites. *Journal of the American Ceramic Society* **92**(2), 323-30.
- Bose S, Tarafder S (2012) Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: A review. *Acta Biomater* **8**(4), 1401-21.
- Boutsioukis C, Noura G, Lambrianidis T (2008) Ex vivo study of the efficiency of two techniques for the removal of mineral trioxide aggregate used as a root canal filling material. *J Endod* **34**(10), 1239-42.
- Branstetter J, von Fraunhofer JA (1982) The physical properties and sealing action of endodontic sealer cements: A review of the literature. *J Endod* **8**(7), 312-6.
- Brasil DS, Soares JA, Horta MC (2010) Periapical repair in dog teeth: Root canal adhesive filling by using the resilon system. *J Endod* **36**(3), 482-8.
- Brayton SM, Davis SR, Goldman M (1973) Gutta-percha root canal fillings. An in vitro analysis. I. *Oral Surg Oral Med Oral Pathol* **35**(2), 226-31.
- Brydson JA (1999) Polyethylene, plastics materials. pp. 205–46. Oxford: Butterworth-Heinemann.
- Bryington MS, Hayashi M, Kozai Y et al. (2013) The influence of nano hydroxyapatite coating on osseointegration after extended healing periods. *Dent Mater* **29**(5), 514-20.
- Buchanan LS (2009) *Common misconceptions about carrier-based obturation. Endodontic practice*, pp. 30-34.
- Burtscher P (1993) Stability of radicals in cured composite materials. *Dent Mater* **9**(4), 218-21.

- Bystrom A, Claesson R, Sundqvist G (1985) The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. *Endod Dent Traumatol* **1**(5), 170-5.
- Bystrom A, Sundqvist G (1981) Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand J Dent Res* **89**(4), 321-8.
- Camps J, Pommel L, Bukiet F, About I (2004) Influence of the powder/liquid ratio on the properties of zinc oxide-eugenol-based root canal sealers. *Dental Material* **20**(10), 915-23.
- Caplan DJ, Kolker J, Rivera EM, Walton RE (2002) Relationship between number of proximal contacts and survival of root canal treated teeth. *Int Endod J* **35**(2), 193-9.
- Carvalho RM, Pereira JC, Yoshiyama M, Pashley DH (1996) A review of polymerization contraction: The influence of stress development versus stress relief. *Oper Dent* **21**(1), 17-24.
- Castaldini A, Cavallini A (1986) "Creep behaviour of composite bone cement". In: Christel P, Meunier A, Lee A, eds. *Biological and Biomedical Performance of Biomaterials*, pp. 525-30. Amsterdam: Elsevier.
- Chandrasekhar V, Morishetty PK, Metla SL, Raju RV (2011) Expansion of gutta-percha in contact with various concentrations of zinc oxide-eugenol sealer: A three-dimensional volumetric study. *J Endod* **37**(5), 697-700.
- Chen CL, Huang TH, Ding SJ, Shie MY, Kao CT (2009) Comparison of calcium and silicate cement and mineral trioxide aggregate biologic effects and bone markers expression in mg63 cells. *J Endod* **35**(5), 682-5.
- Chen GQ, Wu Q (2005) The application of polyhydroxyalkanoates as tissue engineering materials. *Biomaterials* **26**(33), 6565-78.
- Chen H, Teixeira FB, Ritter AL, Levin L, Trope M (2008) The effect of intracanal anti-inflammatory medicaments on external root resorption of replanted dog teeth after extended extra-oral dry time. *Dent Traumatol* **24**(1), 74-8.
- Chueh LH, Ho YC, Kuo TC, Lai WH, Chen YH, Chiang CP (2009) Regenerative endodontic treatment for necrotic immature permanent teeth. *J Endod* **35**(2), 160-4.
- Chung K, Lin T, Wang F (1998) Flexural strength of a provisional resin material with fibre addition. *J Oral Rehabil* **25**(3), 214-7.
- Clark DS, ElDeeb ME (1993) Apical sealing ability of metal versus plastic carrier thermafil obturators. *J Endod* **19**(1), 4-9.
- Clinton K, Van Himel T (2001) Comparison of a warm gutta-percha obturation technique and lateral condensation. *J Endod* **27**(11), 692-5.

- Cobankara FK, Ungor M, Belli S (2002) The effect of two different root canal sealers and smear layer on resistance to root fracture. *J Endod* **28**(8), 606-9.
- Cohen S, Hargreaves K (2006) Pathways of the pulp. 9th edn. St Louis: Mosby-Elsevier
- Collins J, Walker MP, Kulild J, Lee C (2006) A comparison of three gutta-percha obturation techniques to replicate canal irregularities. *J Endod* **32**(8), 762-5.
- Combe E, Burke F, Douglas W (1999) Dental biomaterials. USA: Kluwer Academic publishers.
- Combe EC, Cohen BD, Cummings K (2001) Alpha- and beta-forms of gutta-percha in products for root canal filling. *International Endodontic Journal* **34**(6), 447-51.
- Cordell JM, Vogl ML, Wagoner Johnson AJ (2009) The influence of micropore size on the mechanical properties of bulk hydroxyapatite and hydroxyapatite scaffolds. *J Mech Behav Biomed Mater* **2**(5), 560-70.
- Cramer NB, Stansbury JW, Bowman CN (2011) Recent advances and developments in composite dental restorative materials. *J Dent Res* **90**(4), 402-16.
- Cury JA, Tenuta LMA (2009) Enamel remineralization: Controlling the caries disease or treating early caries lesions. *Braz Oral Res* **23**, 23-30.
- Cvek M (1992) Prognosis of luxated non-vital maxillary incisors treated with calcium hydroxide and filled with gutta-percha. A retrospective clinical study. *Endod Dent Traumatol* **8**(2), 45-55.
- Da Silva D, Endal U, Reynaud A, Portenier I, Ørstavik D, Haapasalo M (2002) A comparative study of lateral condensation, heat-softened gutta-percha, and a modified master cone heat-softened backfilling technique. *Int Endod J* **35**(12), 1005-11.
- da Silva Neto UX, de Moraes IG, Westphalen VP, Menezes R, Carneiro E, Fariniuk LF (2007) Leakage of 4 resin-based root-canal sealers used with a single-cone technique. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **104**(2), e53-7.
- Dagang G, Haoliang S, Kewei X, Yong H (2007) Long-term variations in mechanical properties and in vivo degradability of cpc/plga composite. *J Biomed Mater Res B Appl Biomater* **82**(2), 533-44.
- Daniel RK, Olding M (1984) An absorbable anastomotic device for microvascular surgery: Clinical applications. *Plast Reconstr Surg* **74**(3), 337-42.
- Darvell BW, Wu RC (2011) "Mta"- an hydraulic silicate cement: Review update and setting reaction. *Dent Mater* **27**(5), 407-22.
- De-Deus G, Namen F, Galan J (2008a) Reduced long-term sealing ability of adhesive root fillings after water-storage stress. *J Endod* **34**(3), 322-5.
- De-Deus G, Reis C, Beznos D, de Abranches AM, Coutinho-Filho T, Paciornik S (2008b) Limited ability of three commonly used thermoplasticized gutta-percha techniques in filling oval-shaped canals. *J Endod* **34**(11), 1401-5.

- de Groot K, Geesink R, Klein CP, Serekian P (1987) Plasma sprayed coatings of hydroxylapatite. *J Biomed Mater Res* **21**(12), 1375-81.
- De Moor RJ, Hommez GM (2002) The long-term sealing ability of an epoxy resin root canal sealer used with five gutta percha obturation techniques. *Int Endod J* **35**(3), 275-82.
- De Santis R, Ambrosio L, Nicolais L (2000) Polymer-based composite hip prostheses. *J Inorg Biochem* **79**(1-4), 97-102.
- de Souza Filho FJ, Gallina G, Gallottini L, Russo R, Cumbo EM (2012) Innovations in endodontic filling materials: Guttapercha vs resilon. *Curr Pharm Des* **18**(34), 5553-8.
- Deb S, Wang M, Tanner KE, Bonfield W (1996) Hydroxyapatite-polyethylene composites: Effect of grafting and surface treatment of hydroxyapatite. *Journal of Materials Science: Materials in Medicine* **7**(4), 191-3.
- Di Silvio L, Dalby M, Bonfield W (2002) Osteoblast behaviour on ha/pe composite surfaces with different ha volumes. *Biomaterials* **23**(1), 101-7.
- do Nascimento C, Issa JP, Lyomasa MM et al. (2008) Bone repair using mineral trioxide aggregate combined to a material carrier, associated or not with calcium hydroxide in bone defects. *Micron* **39**(7), 868-74.
- Dowker SEP, Davis GR, Elliott JC (1997) X-ray microtomography: Nondestructive three-dimensional imaging for in vitro endodontic studies. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **83**(4), 510-6.
- Duarte MA, Demarchi AC, Yamashita JC, Kuga MC, Fraga Sde C (2003) Ph and calcium ion release of 2 root-end filling materials. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **95**(3), 345-7.
- Duggan D, Arnold RR, Teixeira FB, Caplan DJ, Tawil P (2009) Periapical inflammation and bacterial penetration after coronal inoculation of dog roots filled with realseal 1 and thermafil. *J Endod* **35**(6), 852-7.
- Edlund U, Albertsson AC (2003) Polyesters based on diacid monomers. *Adv Drug Deliv Rev* **55**(4), 585-609.
- Eick JD, Robinson SJ, Byerley TJ, Chappelow CC (1993) Adhesives and nonshrinking dental resins of the future. *Quintessence Int* **24**(9), 632-40.
- Ekstrand K, Ruyter IE, Wellendorf H (1987) Carbon/graphite fiber reinforced poly(methyl methacrylate): Properties under dry and wet conditions. *J Biomed Mater Res* **21**(9), 1065-80.
- El-Ma'aitha AM, Qualtrough AJ, Watts DC (2012) A micro-computed tomography evaluation of mineral trioxide aggregate root canal fillings. *J Endod* **38**(5), 670-2.

- Eldeniz AU, Erdemir A, Belli S (2005) Effect of edta and citric acid solutions on the microhardness and the roughness of human root canal dentin. *J Endod* **31**(2), 107-10.
- Eligio T, Rieumont J, Sanchez R, Silva JFS (1999) Characterization of chemically modified poly(3-hydroxyalkanoates) and their performance as matrix for hormone release. *Die Angewandte makromolekulare Chemie*(270), 69-75.
- Enkel B, Dupas C, Armengol V et al. (2008) Bioactive materials in endodontics. *Expert Rev Med Devices* **5**(4), 475-94.
- Epley SR, Fleischman J, Hartwell G, Cicalese C (2006) Completeness of root canal obturations: Epiphany techniques versus gutta-percha techniques. *J Endod* **32**(6), 541-4.
- Estrela C, Sydney GB, Bammann LL, Felipe Junior O (1995) Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. *Braz Dent J* **6**(2), 85-90.
- European Society of Endodontology Guidelines (2006) Quality guidelines for endodontic treatment: Consensus report of the european society of endodontology. *Int Endod J* **39**(12), 921-30.
- Evans SL, Gregson PJ (1998) Composite technology in load-bearing orthopaedic implants. *Biomaterials* **19**(15), 1329-42.
- Fang Z, Feng Q, Tan R (2013) In-situ grown hydroxyapatite whiskers reinforced porous ha bioceramic. *Ceramics International* **39**(8), 8847-52.
- Favieri A, Campos LC, Burity VH, Santa Cecilia M, Abad Eda C (2008) Use of biomaterials in periradicular surgery: A case report. *J Endod* **34**(4), 490-4.
- Ferrari M, Mason PN, Goracci C, Pashley DH, Tay FR (2004) Collagen degradation in endodontically treated teeth after clinical function. *J Dent Res* **83**(5), 414-9.
- Ferreira JJ, Rhodes JS, Pitt Ford TR (2001) The efficacy of gutta-percha removal using profiles. *Int Endod J* **34**(4), 267-74.
- Fiore GL, Jing F, Young JVG, Cramer CJ, Hillmyer MA (2010) High tg aliphatic polyesters by the polymerization of spirolactide derivatives. *Polymer Chemistry* **1**(6), 870-7.
- Fransen JN, He J, Glickman GN, Rios A, Shulman JD, Honeyman A (2008) Comparative assessment of activ gp/glass ionomer sealer, resilon/epiphany, and gutta-percha/ah plus obturation: A bacterial leakage study. *J Endod* **34**(6), 725-7.
- Fridland M, Rosado R (2005) Mta solubility: A long term study. *J Endod* **31**(5), 376-9.
- Friedman S, Abitbol S, Lawrence HP (2003) Treatment outcome in endodontics: The toronto study. Phase 1: Initial treatment. *Journal of Endodontics* **29**(12), 787-93.
- Friedrich K, Karsch U (1983) Failure processes in particulate filled polypropylene. *Fibre Sci. Technol* **18**(1), 37-52.

- Furukawa T, Sato H, Murakami R et al. (2007) Comparison of miscibility and structure of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)/poly(l-lactic acid) blends with those of poly(3-hydroxybutyrate)/poly(l-lactic acid) blends studied by wide angle x-ray diffraction, differential scanning calorimetry, and ftir microspectroscopy. *Polymer* **48**(6), 1749-55.
- Gandolfi MG, Sauro S, Mannocci F et al. (2007) New tetrasilicate cements as retrograde filling material: An in vitro study on fluid penetration. *J Endod* **33**(6), 742-5.
- Gatewood RS, Parsell DE, Rushing CC (2004) Cross-sectional assessment of apical dye penetration following clinical simulation of various endodontic techniques. *Gen Dent* **52**(4), 342-7; quiz 8, 65-6.
- Genc O, Alacam T, Kayaoglu G (2011) Evaluation of three instrumentation techniques at the precision of apical stop and apical sealing of obturation. *J Appl Oral Sci* **19**(4), 350-4.
- Gencoglu N (2003) Comparison of 6 different gutta-percha techniques (part ii): Thermafil, js quick-fill, soft core, microseal, system b, and lateral condensation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **96**(1), 91-5.
- Gencoglu N, Garip Y, Bas M, Samani S (2002) Comparison of different gutta-percha root filling techniques: Thermafil, quick-fill, system b, and lateral condensation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **93**(3), 333-6.
- Gesi A, Raffaelli O, Goracci C, Pashley DH, Tay FR, Ferrari M (2005) Interfacial strength of resilon and gutta-percha to intraradicular dentin. *J Endod* **31**(11), 809-13.
- Giachetti L, Scaminaci Russo D, Baldini M, Bertini F, Steier L, Ferrari M (2012) Push-out strength of translucent fibre posts cemented using a dual-curing technique or a light-curing self-adhering material. *Int Endod J* **45**(3), 249-56.
- Gillen BM, Looney SW, Gu LS et al. (2011) Impact of the quality of coronal restoration versus the quality of root canal fillings on success of root canal treatment: A systematic review and meta-analysis. *J Endod* **37**(7), 895-902.
- Gonzales JR, Rodekirchen H (2007) Endodontic and periodontal treatment of an external cervical resorption. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **104**(1), e70-7.
- Goodman A, Schilder H, Aldrich W (1974) The thermomechanical properties of gutta-percha. li. The history and molecular chemistry of gutta-percha. *Oral Surg Oral Med Oral Pathol* **37**(6), 954-61.
- Göpferich A (1996) Mechanisms of polymer degradation and erosion. *Biomaterials* **17**(2), 103-14.
- Gopikrishna V, Parameswaren A (2006) Coronal sealing ability of three sectional obturation techniques--simplifill, thermafil and warm vertical compaction--

- compared with cold lateral condensation and post space preparation. *Aust Endod J* **32**(3), 95-100.
- Grande NM, Plotino G, Lavorgna L et al. (2007) Influence of different root canal-filling materials on the mechanical properties of root canal dentin. *J Endod* **33**(7), 859-63.
- Griffin GJL (1993) *Chemistry and technology of biodegradable polymers*, London; New York; New York: Blackie Academic & Professional ; Chapman & Hall.
- Grigoratos D, Knowles J, Ng YL, Gulabivala K (2001) Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. *Int Endod J* **34**(2), 113-9.
- Grossman L (1988) *Endodontics*. 11 edn. Philadelphia: Lea & Febiger.
- Guida A, Towler MR, Wall JG, Hill RG, Eramo S (2003) Preliminary work on the antibacterial effect of strontium in glass ionomer cements. *Journal of Materials Science Letters* **22**(20), 1401-3.
- Gulabivala K, Holt R, Long B (1998) An in vitro comparison of thermoplasticised gutta-percha obturation techniques with cold lateral condensation. *Endod Dent Traumatol* **14**(6), 262-9.
- Gutarts R, Nusstein J, Reader A, Beck M (2005) In vivo debridement efficacy of ultrasonic irrigation following hand-rotary instrumentation in human mandibular molars. *J Endod* **31**(3), 166-70.
- Gutmann J, Dumsha T, Lovdahl P (2006) *Problem solving in endodontics*, 4th edn; St Louis, MO: Elsevier.
- Gutmann J, Witherspoon D (2002) Obturation of the cleaned and shaped root canal system. In: Cohen S & Burns R, ed. *Pathways of the pulp*, 8th edn; pp. 293-364: St Louis, MO: CV Mosby.
- Gutmann JL, Saunders WP, Saunders EM, Nguyen L (1993) A assessment of the plastic thermafil obturation technique. Part 1. Radiographic evaluation of adaptation and placement. *Int Endod J* **26**(3), 173-8.
- Haapasalo M, Shen Y, Qian W, Gao Y (2010) Irrigation in endodontics. *Dent Clin North Am* **54**(2), 291-312.
- Habraken WJ, Wolke JG, Jansen JA (2007) Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering. *Adv Drug Deliv Rev* **59**(4-5), 234-48.
- Hakki SS, Bozkurt SB, Hakki EE, Belli S (2009) Effects of mineral trioxide aggregate on cell survival, gene expression associated with mineralized tissues, and biomineralization of cementoblasts. *J Endod* **35**(4), 513-9.

- Hammad M, Qualtrough A, Silikas N (2008) Three-dimensional evaluation of effectiveness of hand and rotary instrumentation for retreatment of canals filled with different materials. *J Endod* **34**(11), 1370-3.
- Hammad M, Qualtrough A, Silikas N (2009) Evaluation of root canal obturation: A three-dimensional in vitro study. *J Endod* **35**(4), 541-4.
- Han L, Okiji T (2011) Uptake of calcium and silicon released from calcium silicate-based endodontic materials into root canal dentine. *Int Endod J* **44**(12), 1081-7.
- Hancox N (1982) "High performance composites with resin matrices". In: Kelly A, Mileiko S, eds. *Handbook of Composites, Vol 4. Fabrication of Composites*, pp. 1-44. North Holland, Amsterdam.
- Hardy TG, Jr., Pace WG, Maney JW, Katz AR, Kaganov AL (1985) A biofragmentable ring for sutureless bowel anastomosis. An experimental study. *Dis Colon Rectum* **28**(7), 484-90.
- Hatamleh MM, Watts DC (2011) Porosity and color of maxillofacial silicone elastomer. *J Prosthodont* **20**(1), 60-6.
- Hayakawa T, Tomita F, Okiji T (2010) Influence of the diameter and taper of root canals on the removal efficiency of thermafil plus plastic carriers using protaper retreatment files. *J Endod* **36**(10), 1676-8.
- Heling I, Chandler NP (1996) The antimicrobial effect within dentinal tubules of four root canal sealers. *J Endod* **22**(5), 257-9.
- Hench L (1998) Bioceramics. *Journal of the american ceramic society*. **81**(7), 1705-28.
- Hench L, Andersson O (1993) "Bioactive glasses". In: Hench LL, Wilso J, eds. *An introduction to bioceramics*, pp. 41–62. Singapore: World Scientific Publishing Company.
- Hench L, Splinter R, Allen W, Greenlee T (1971) Bonding mechanisms at the interface of ceramic prosthetic materials. *J. Biomed. Mater. Res. Symp. 2 (Part I)*, 117-41.
- Hench L, Wheeler D, Greenspan D (1998) Molecular control of bioactivity in sol-gel glasses. *Journal of Sol-Gel Science and Technology* **13**(1-3), 245-50.
- Hench LL, Paschall HA (1973) Direct chemical bond of bioactive glass-ceramic materials to bone and muscle. *J Biomed Mater Res* **7**(3), 25-42.
- Hench LL, Xynos ID, Polak JM (2004) Bioactive glasses for in situ tissue regeneration. *J Biomater Sci Polym Ed* **15**(4), 543-62.
- Hess D, Solomon E, Spears R, He J (2011) Retreatability of a bioceramic root canal sealing material. *J Endod* **37**(11), 1547-9.
- Higashi S, Yamamuro T, Nakamura T, Ikada Y, Hyon SH, Jamshidi K (1986) Polymer-hydroxyapatite composites for biodegradable bone fillers. *Biomaterials* **7**(3), 183-7.

- Hirai VH, da Silva Neto UX, Westphalen VPD, Perin CP, Carneiro E, Fariniuk LF (2010) Comparative analysis of leakage in root canal filling performed with gutta-percha and resilon cones with ah plus and epiphany sealers. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **109**(2), e131-5.
- Hiraishi N, Papacchini F, Loushine RJ (2005) Shear bond strength of resilon to a methacrylate-based root canal sealer. *Int Endod J* **38**(10), 753-63.
- Holland R, de Souza V, Nery MJ et al. (2002) Calcium salts deposition in rat connective tissue after the implantation of calcium hydroxide-containing sealers. *J Endod* **28**(3), 173-6.
- Hollanda AC, Estrela CR, Decurcio Dde A, Silva JA, Estrela C (2009) Sealing ability of three commercial resin-based endodontic sealers. *Gen Dent* **57**(4), 368-73.
- Holt R, Jr. (1986) Dental implants. *J West Soc Periodontol Periodontal Abstr* **34**(2), 49-55.
- Hoskinson SE, Ng YL, Hoskinson AE, Moles DR, Gulabivala K (2002) A retrospective comparison of outcome of root canal treatment using two different protocols. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **93**(6), 705-15.
- Hsieh K-H, Liao K-H, Lai EH-H, Lee B-S, Lee C-Y, Lin C-P (2008) A novel polyurethane-based root canal-obturation material and urethane acrylate-based root canal sealer—part i: Synthesis and evaluation of mechanical and thermal properties. *J Endod* **34**(3), 303-5.
- <http://www.britannica.com/EBchecked/media/125939/The-linear-form-of-polyethylene-known-as-high-density-polyethylene> (Retrieved 27 January 2014) Low and high-density polyethylene: Branch and linear forms of polyethylene. Encyclopædia Britannica Online.
- Hu S, Chang J, Liu M, Ning C (2009) Study on antibacterial effect of 45s5 bioglass®. *Journal of Materials Science: Materials in Medicine* **20**(1), 281-6.
- Huang J, Di Silvio L, Wang M, Rehman I, Ohtsuki C, Bonfield W (1997) Evaluation of in vitro bioactivity and biocompatibility of bioglass-reinforced polyethylene composite. *J Mater Sci Mater Med* **8**(12), 809-13.
- Huang TJG, Schilder H, Nathanson D (1992) Effects of moisture content and endodontic treatment on some mechanical properties of human dentin. *J Endod* **18**(5), 209-15.
- Ibarrola JL, Knowles KI, Ludlow MO (1993) Retrievability of thermafil plastic cores using organic solvents. *J Endod* **19**(8), 417-8.
- Ikram OH, Patel S, Sauro S, Mannocci F (2009) Micro-computed tomography of tooth tissue volume changes following endodontic procedures and post space preparation. *Int Endod J* **42**(12), 1071-6.

- Imai Y, Komabayashi T (2003) Properties of a new injectable type of root canal filling resin with adhesiveness to dentin. *J Endod* **29**(1), 20-3.
- Imura N, Pinheiro ET, Gomes BP, Zaia AA, Ferraz CC, Souza-Filho FJ (2007) The outcome of endodontic treatment: A retrospective study of 2000 cases performed by a specialist. *J Endod* **33**(11), 1278-82.
- Inan U, Aydemir H, Tasdemir T (2007) Leakage evaluation of three different root canal obturation techniques using electrochemical evaluation and dye penetration evaluation methods. *Aust Endod J* **33**(1), 18-22.
- Islam I, Chng HK, Yap AU (2006) Comparison of the physical and mechanical properties of mta and portland cement. *J Endod* **32**(3), 193-7.
- Jacobsen EL, BeGole EA, Vitkus DD, Daniel JC (1987) An evaluation of two newly formulated calcium hydroxide cements: A leakage study. *J Endod* **13**(4), 164-9.
- Jacobson HL, Xia T, Baumgartner JC, Marshall JG, Beeler WJ (2002) Microbial leakage evaluation of the continuous wave of condensation. *J Endod* **28**(4), 269-71.
- Jalalzadeh SM, Mamavi A, Khoshbin E, Karapanou V, Mohammadi Z (2010) The effect of post space preparation in teeth obturated with mineral trioxide aggregate evaluated using a fluid filtration system. *J Oral Sci* **52**(4), 567-70.
- Jameson MW, Hood JA, Tidmarsh BG (1993) The effects of dehydration and rehydration on some mechanical properties of human dentine. *J Biomech* **26**(9), 1055-65.
- Johnson WB (1978) A new gutta-percha technique. *J Endod* **4**(6), 184-8.
- Johnson WB, Kulild JC (2010) Obturation of the cleaned and shaped root canal system. Chapter 10. In: Hargreaves KM, Cohen S, eds. *Cohen's pathways of the pulp*, 10th edn; pp. 349-88. St Louis: Mosby.
- Jones JR, Ehrenfried LM, Hench LL (2006) Optimising bioactive glass scaffolds for bone tissue engineering. *Biomaterials* **27**(7), 964-73.
- Joseph R, McGregor WJ, Martyn MT, Tanner KE, Coates PD (2002) Effect of hydroxyapatite morphology/surface area on the rheology and processability of hydroxyapatite filled polyethylene composites. *Biomaterials* **23**(21), 4295-302.
- Juhasz JA, Best SM, Kawashita M et al. (2003) Bonding strength of the apatite layer formed on glass-ceramic apatite-wollastonite–polyethylene composites. *Journal of Biomedical Materials Research Part A* **67A**(3), 952-9.
- Jung IY, Lee SJ, Hargreaves KM (2008) Biologically based treatment of immature permanent teeth with pulpal necrosis: A case series. *J Endod* **34**(7), 876-87.
- Jung M, Lommel D, Klimek J (2005) The imaging of root canal obturation using micro-ct. *Int Endod J* **38**(9), 617-26.
- Kakehashi S, Stanley HR, Fitzgerald RJ (1965) The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surg Oral Med Oral Pathol* **20**, 340-9.

- Kasuga T (2005) Bioactive calcium pyrophosphate glasses and glass-ceramics. *Acta Biomater* **1**(1), 55-64.
- Kato H, Nakagawa K (2010) Fp core carrier technique: Thermoplasticized gutta-percha root canal obturation technique using polypropylene core. *Bull Tokyo Dent Coll* **51**(4), 213-20.
- Kazandag MK, Tanalp J, Bayrak OF, Sunay H, Bayirh G (2010) Microleakage of various root filling systems by glucose filtration analysis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **109**(6), e96-102.
- Khan YM, Katti DS, Laurencin CT (2004) Novel polymer-synthesized ceramic composite-based system for bone repair: An in vitro evaluation. *J Biomed Mater Res A* **69**(4), 728-37.
- Kinney JH, Balooch M, Marshall GW, Marshall SJ (1999) A micromechanics model of the elastic properties of human dentine. *Arch Oral Biol* **44**(10), 813-22.
- Kirkevang LL, Ørstavik D, Horsted-Bindslev P, Wenzel A (2000) Periapical status and quality of root fillings and coronal restorations in a danish population. *Int Endod J* **33**(6), 509-15.
- Kishen A (2006) Mechanisms and risk factors for fracture predilection in endodontically treated teeth. *Endodontic Topics* **13**(1), 57-83.
- Kishen A, Asundi A (2005) Experimental investigation on the role of water in the mechanical behavior of structural dentine. *J Biomed Mater Res A* **73**(2), 192-200.
- Knowles JC, Hastings GW, Ohta H, Niwa S, Boeree N (1992) Development of a degradable composite for orthopaedic use: In vivo biomechanical and histological evaluation of two bioactive degradable composites based on the polyhydroxybutyrate polymer. *Biomaterials* **13**(8), 491-6.
- Kocak MM, Er O, Saglam BC, Yaman S (2008) Apical leakage of epiphany root canal sealer combined with different master cones. *Eur J Dent* **2**(2), 91-5.
- Kokubo T, Kim HM, Kawashita M (2003) Novel bioactive materials with different mechanical properties. *Biomaterials* **24**(13), 2161-75.
- Koller G, Roether J, Bruce K, Deb S (2008) Antimicrobial potential of bioactive bone cements. *J Appl Biomater Biomech* **6**(1), 16-22.
- Kolluri O, Kaplan S, Rose P (1988) Gas plasma and the treatment of advanced fibers. *Paper presented at the Society of Plastics Engineers Advanced Polymer Composites '88 Technical Conference*.
- Kontakiotis EG, Tzanetakis GN, Loizides AL (2007) A 12-month longitudinal in vitro leakage study on a new silicon-based root canal filling material (gutta-flow). *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **103**(6), 854-9.
- Kumar PP, Good RR (1986) Accurate and economical intracavitary endocurietherapy in the treatment of uterine cervix cancer. *Strahlenther Onkol* **162**(1), 8-12.

- Kumar RV, Shruthi C (2012) Evaluation of the sealing ability of resin cement used as a root canal sealer: An in vitro study. *J Conserv Dent* **15**(3), 274-7.
- Kytridou V, Gutmann JL, Nunn MH (1999) Adaptation and sealability of two contemporary obturation techniques in the absence of the dentinal smear layer. *Int Endod J* **32**(6), 464-74.
- Labella R, Braden M, Deb S (1994) Novel hydroxyapatite-based dental composites. *Biomaterials* **15**, 1197-200.
- Lam PP, Palamara JE, Messer HH (2005) Fracture strength of tooth roots following canal preparation by hand and rotary instrumentation. *J Endod* **31**(7), 529-32.
- Lang H, Korkmaz Y, Schneider K, Raab WH (2006) Impact of endodontic treatments on the rigidity of the root. *J Dent Res* **85**(4), 364-8.
- Latour RA, Jr., Black J (1993) Development of frp composite structural biomaterials: Fatigue strength of the fiber/matrix interfacial bond in simulated in vivo environments. *J Biomed Mater Res* **27**(10), 1281-91.
- Leal F, De-Deus G, Brandão C, Luna AS, Fidel SR, Souza EM (2011) Comparison of the root-end seal provided by bioceramic repair cements and white mta. *Int Endod J* **44**(7), 662-8.
- Leduc J, Fishelberg G (2003) Endodontic obturation: A review. *Gen Dent* **51**(3), 232-3.
- Lee AH, Cheung GS, Wong MC (2012) Long-term outcome of primary non-surgical root canal treatment. *Clin Oral Investig* **16**(6), 1607-17.
- Lee BS, Lai EH, Liao KH, Lee CY, Hsieh KH, Lin CP (2008) A novel polyurethane-based root canal-obturation material and urethane-acrylate-based root canal sealer-part 2: Evaluation of push-out bond strengths. *J Endod* **34**(5), 594-8.
- Lee CQ, Chang Y, Cobb CM, Robinson S, Hellmuth EM (1997) Dimensional stability of thermosensitive gutta-percha. *J Endod* **23**(9), 579-82.
- Lee KW, Williams MC, Camps JJ, Pashley DH (2002a) Adhesion of endodontic sealers to dentin and gutta-percha. *J Endod* **28**(10), 684-8.
- Lee SN, Lee MY, Park WH (2002b) Thermal stabilization of poly(3-hydroxybutyrate) by poly(glycidyl methacrylate). *Journal of Applied Polymer Science* **83**(13), 2945-52.
- LeGeros RZ (1991) Calcium phosphates in oral biology and medicine. *Monogr Oral Sci* **15**, 1-201.
- Leonardo MR, Barnett F, Debelian GJ, de Pontes Lima RK, Bezerra da Silva LA (2007) Root canal adhesive filling in dogs' teeth with or without coronal restoration: A histopathological evaluation. *J Endod* **33**(11), 1299-303.
- Lertchirakarn V, Timyam A, Messer HH (2002) Effects of root canal sealers on vertical root fracture resistance of endodontically treated teeth. *J Endod* **28**(3), 217-9.
- Levitan ME, Himel VT, Luckey JB (2003) The effect of insertion rates on fill length and adaptation of a thermoplasticized gutta-percha technique. *J Endod* **29**(8), 505-8.

- Li G-h, Niu L-n, Selem LC et al. (2014a) Quality of obturation achieved by an endodontic core-carrier system with crosslinked gutta-percha carrier in single-rooted canals. *J Dent*, doi: 10.1016/j.jdent.2014.04.008 [Article in Press].
- Li GH, Niu LN, Zhang W et al. (2014b) Ability of new obturation materials to improve the seal of the root canal system: A review. *Acta Biomater* **10**(3), 1050-63.
- Limkangwalmongkol S, Abbott PV, Sandler AB (1992) Apical dye penetration with four root canal sealers and gutta-percha using longitudinal sectioning. *J Endod* **18**(11), 535-9.
- Lipski M, Woźniak K (2003) In vitro infrared thermographic assessment of root surface temperature rises during thermafil retreatment using system b. *J Endod* **29**(6), 413-5.
- Loewenstein WR, Rathkamp R (1955) A study on the pressoreceptive sensibility of the tooth. *J Dent Res* **34**(2), 287-94.
- Lootz D, Behrend D, Kramer S et al. (2001) Laser cutting: Influence on morphological and physicochemical properties of polyhydroxybutyrate. *Biomaterials* **22**(18), 2447-52.
- Lorbach O, Wilmes P, Brogard P, Seil R (2008) Complications related to implants in arthroscopic shoulder surgery. *Orthopade* **37**(11), 1073-9.
- Loushine BA, Bryan TE, Looney SW et al. (2011) Setting properties and cytotoxicity evaluation of a premixed bioceramic root canal sealer. *J Endod* **37**(5), 673-7.
- Love RM, Jenkinson HF (2002) Invasion of dentinal tubules by oral bacteria. *Crit Rev Oral Biol Med* **13**(2), 171-83.
- Luo Q, Hiessl S, Poehlein A, Steinbuchel A (2013) Microbial gutta-percha degradation shares common steps with rubber degradation by nocardia nova sh22a. *Appl Environ Microbiol* **79**(4), 1140-9.
- Mackinnon SE, Dellon AL, Hudson AR, Hunter DA (1985) Nerve regeneration through a pseudosynovial sheath in a primate model. *Plast Reconstr Surg* **75**(6), 833-41.
- Madera-Santana T, Robledo D, Azamar J, Rios-Soberanis, Freile-Pelegrin Y (2010) Preparation and characterization of low density polyethylene-agar biocomposites: Torque-rheological, mechanical, thermal and morphological properties. *Polym Eng Sci*, 585–91.
- Madison S, Swanson K, Chiles SA (1987) An evaluation of coronal microleakage in endodontically treated teeth. Part ii. Sealer types. *Journal of Endodontics* **13**(3), 109-12.
- Magalhaes BS, Johann JE, Lund RG, Martos J, Del Pino FA (2007) Dissolving efficacy of some organic solvents on gutta-percha. *Braz Oral Res* **21**(4), 303-7.
- Malinova L, Brozek J (2011) Mixtures poly((r)-3-hydroxybutyrate) and poly(l-lactic acid) subjected to dsc. *Journal of Thermal Analysis and Calorimetry* **103**(2), 653-60.

- Maniglia-Ferreira C, Bonecker G, Silva JB, Jr., de Paula RC, Feitosa JP, Souza-Filho FJ (2008) Degradation of trans-polyisoprene after root filling with thermoplasticized techniques. *Int Endod J* **41**(4), 296-302.
- Maniglia-Ferreira C, Silva JB, Jr., de Paula RC et al. (2007) Degradation of trans-polyisoprene over time following the analysis of root fillings removed during conventional retreatment. *International Endodontic Journal* **40**(1), 25-30.
- Mannocci F, Bhuva B, Stern S (2008) Restoring teeth following root canal re-treatment. *Endodontic Topics* **19**(1), 125-52.
- Mannocci F, Innocenti M, Ferrari M (1998) Stereomicroscopic and scanning electron microscopic study of roots obturated with vertically condensed gutta-percha, epoxy resin cement, and dentin bonding agent. *J Endod* **24**(6), 397-400.
- Marending M, Bubenhofer SB, Sener B, De-Deus G (2013) Primary assessment of a self-adhesive gutta-percha material. *Int Endod J* **46**(4), 317-22.
- Marshall GW, Jr., Marshall SJ, Kinney JH, Balooch M (1997) The dentin substrate: Structure and properties related to bonding. *J Dent* **25**(6), 441-58.
- Masuelli MA (2013) *Introduction of fibre-reinforced polymers – polymers and composites: Concepts, properties and processes. Chapter 1, pp 1-40. Creative commons corporation.*
- McCrum N, Buckley C, Bucknall C (1997) Principles of polymer engineering. UK: Oxford University Press, Incorporated.
- Meire M, De Moor R (2008) Mineral trioxide aggregate repair of a perforating internal resorption in a mandibular molar. *J Endod* **34**(2), 220-3.
- Meyer KM, Kollmar F, Schirrmeister JF, Schneider F, Hellwig E (2006) Analysis of shrinkage of different gutta-percha types using optical measurement methods. *Schweiz Monatsschr Zahnmed* **116**(4), 356-61.
- Michaud RA, Burgess J, Barfield RD, Cakir D, McNeal SF, Eleazer PD (2008) Volumetric expansion of gutta-percha in contact with eugenol. *Journal of Endodontics* **34**, 1528-32.
- Min KS, Park HJ, Lee SK et al. (2008) Effect of mineral trioxide aggregate on dentin bridge formation and expression of dentin sialoprotein and heme oxygenase-1 in human dental pulp. *J Endod* **34**(6), 666-70.
- Misra SK, Valappil SP, Roy I, Boccaccini AR (2006) Polyhydroxyalkanoate (pha)/inorganic phase composites for tissue engineering applications. *Biomacromolecules* **7**(8), 2249-58.
- Mohammadi MS, Ahmed I, Muja N, Rudd CD, Bureau MN, Nazhat SN (2011) Effect of phosphate-based glass fibre surface properties on thermally produced poly(lactic acid) matrix composites. *J Mater Sci Mater Med* **22**(12), 2659-72.

- Mohn D, Bruhin C, Luechinger NA, Stark WJ, Imfeld T, Zehnder M (2010) Composites made of flame-sprayed bioactive glass 45s5 and polymers: Bioactivity and immediate sealing properties. *Int Endod J* **43**(11), 1037-46.
- Mohsen NM, Craig RG (1995) Effect of silanation of fillers on their dispersability by monomer systems. *J Oral Rehabil* **22**(3), 183-9.
- Molven O, Halse A (1988) Success rates for gutta-percha and kloroperka n-0 root fillings made by undergraduate students: Radiographic findings after 10-17 years. *Int Endod J* **21**(4), 243-50.
- Monteiro Bramante C, Demarchi AC, de Moraes IG et al. (2008) Presence of arsenic in different types of mta and white and gray portland cement. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **106**(6), 909-13.
- Monticelli F, Sadek FT, Schuster GS et al. (2007) Efficacy of two contemporary single-cone filling techniques in preventing bacterial leakage. *J Endod* **33**(3), 310-3.
- Moura-Netto C, Pinto T, Davidowicz H, de Moura AAM (2009) Apical leakage of three resin-based endodontic sealers after 810-nm-diode laser irradiation. *Photomed Laser Surg* **27**(6), 891-4.
- Mourino V, Cattalini JP, Roether JA, Dubey P, Roy I, Boccaccini AR (2013) Composite polymer-bioceramic scaffolds with drug delivery capability for bone tissue engineering. *Expert Opin Drug Deliv* **10**(10), 1353-65.
- Nair PN (2004) Pathogenesis of apical periodontitis and the causes of endodontic failures. *Crit Rev Oral Biol Med* **15**(6), 348-81.
- Nair PNR (2006) On the cause of persistent apical periodontitis: A review. *Int Endod J* **39**(4), 249-81.
- Najibfard K, Ramalingam K, Chedjieu I, Amaechi BT (2011) Remineralization of early caries by a nano-hydroxyapatite dentifrice. *J Clin Dent* **22**(5), 139-43.
- Natera M, Pileggi R, Nair U (2011) A comparison of two gutta-percha obturation techniques to replicate canal irregularities in a split-tooth model. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontology* **112**, e29-34.
- Nawal RR, Parande M, Sehgal R, Rao NR, Naik A (2011) A comparative evaluation of 3 root canal filling systems. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* **111**(3), 387-93.
- Nazhat SN, Joseph R, Wang M, Smith R, Tanner KE, Bonfield W (2000) Dynamic mechanical characterization of hydroxyapatite reinforced polyethylene: Effect of particle size. *J Mater Sci Mater Med* **11**(10), 621-8.
- Nazhat SN, Kellomaki M, Tormala P, Tanner KE, Bonfield W (2001) Dynamic mechanical characterization of biodegradable composites of hydroxyapatite and polylactides. *J Biomed Mater Res* **58**(4), 335-43.

- Ng YL, Mann V, Gulabivala K (2010) Tooth survival following non-surgical root canal treatment: A systematic review of the literature. *Int Endod J* **43**(3), 171-89.
- Ng YL, Mann V, Gulabivala K (2011) A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: Part 1: Periapical health. *Int Endod J* **44**(7), 583-609.
- Ng YL, Mann V, Rahbaran S, Lewsey J, Gulabivala K (2008) Outcome of primary root canal treatment: Systematic review of the literature – part 2. Influence of clinical factors. *Int Endod J* **41**(1), 6-31.
- Ngu J, Akmil N, Chuah L, Choong T, Yaw S (2009) Characterization of mechanical properties: Low density polyethylene composite using α -alumina submicrometer sized particle as filler. *Malaysia Polymer International Conference (MPIC 2009)*. Malaysia National University, Bangi.
- Nielsen RB, Alyassin AM, Peters DD, Carnes DL, Lancaster J (1995) Microcomputed tomography: An advanced system for detailed endodontic research. *J Endod* **21**(11), 561-8.
- Nooshin V (2010) Thermomechanical characterisation of biodegradable polymers for the assessment of their potential use as intracanal medicaments and carriers for carrier-based obturation systems (Master thesis: King's College London Dental Institute).
- Olson AK, Hartwell GR, Weller RN (1989) Evaluation of the controlled placement of injected thermoplasticized gutta-percha. *J Endod* **15**(7), 306-9.
- Onay EO, Ungor M, Orucoglu H (2006) An in vitro evaluation of the apical sealing ability of a new resin based root canal obturation system. *J Endod* **32**(10), 976-8.
- Oosterbos CJ, Vogely H, Nijhof MW et al. (2002) Osseointegration of hydroxyapatite-coated and noncoated ti6al4v implants in the presence of local infection: A comparative histomorphometrical study in rabbits. *J Biomed Mater Res* **60**(3), 339-47.
- Ørstavik D (1996) Time-course and risk analyses of the development and healing of chronic apical periodontitis in man. *Int Endod J* **29**(3), 150-5.
- Ørstavik D (2005) Materials used for root canal obturation: Technical, biological and clinical testing. *Endodontic Topics* **12**(1), 25-38.
- Ørstavik D, Mjor IA (1988) Histopathology and x-ray microanalysis of the subcutaneous tissue response to endodontic sealers. *J Endod* **14**(1), 13-23.
- Ørstavik D, Nordahl I, Tibballs JE (2001) Dimensional change following setting of root canal sealer materials. *Dent Mater* **17**(6), 512-9.
- Ørstavik D, Pitt Ford T (2008) Apical periodontitis: Microbial infection and host responses. In: Ørstavik D, Pitt Ford T, eds. *Essential endodontology; prevention*

- and treatment of apical periodontitis, 2nd edn; pp. 1-9. Kent, UK: Blackwell Munksgaard.
- Paredes-Vieyra J, Enriquez FJJ (2012) Success rate of single- versus two-visit root canal treatment of teeth with apical periodontitis: A randomized controlled trial. *J Endod* **38**(9), 1164-9.
- Parirokh M, Torabinejad M (2010) Mineral trioxide aggregate: A comprehensive literature review--part i: Chemical, physical, and antibacterial properties. *J Endod* **36**(1), 16-27.
- Park DS, Kim IS, Kim H et al. (2010) Improved biocompatibility of hydroxyapatite thin film prepared by aerosol deposition. *J Biomed Mater Res B Appl Biomater* **94**(2), 353-8.
- Parker H, Glickman G (1993) Solubility of plastic thermofil carriers. *Journal of Dental Research* **72**, 188.
- Parra C, Gonzalez G, Albano C (2009) Synthesis and characterization of composite materials hdpe/ha and pmma/ha prepared by sonochemistry. *Macromol Symp*(286), 60–9.
- Pashley DH, Tay FR, Yiu C et al. (2004) Collagen degradation by host-derived enzymes during aging. *J Dent Res* **83**(3), 216-21.
- Pasqualini D, Scotti N, Mollo L (2007) Microbial leakage of gutta-percha and resilon root canal filling material: A comparative study using a new homogenous assay for sequence detection. *J Biomater Appl* **22**(4), 337-52.
- Patel S, Brady E, Wilson R, Brown J, Mannocci F (2013) The detection of vertical root fractures in root filled teeth with periapical radiographs and cbct scans. *Int Endod J* **46**(12), 1140-52.
- Pereira MM, Clark AE, Hench LL (1994) Calcium phosphate formation on sol-gel-derived bioactive glasses in vitro. *Journal of Biomedical Materials Research* **28**(6), 693-8.
- Peter M, Binulal NS, Soumya S et al. (2010) Nanocomposite scaffolds of bioactive glass ceramic nanoparticles disseminated chitosan matrix for tissue engineering applications. *Carbohydrate Polymers* **79**(2), 284-9.
- Peters OA, Barbakow F, Peters CI (2004) An analysis of endodontic treatment with three nickel-titanium rotary root canal preparation techniques. *Int Endod J* **37**(12), 849-59.
- Pettiette MT, Metzger Z, Phillips C, Trope M (1999) Endodontic complications of root canal therapy performed by dental students with stainless-steel k-files and nickel-titanium hand files. *J Endod* **25**(4), 230-34.
- Pitout E, Oberholzer TG, Blignaut E, Molepo J (2006) Coronal leakage of teeth root-filled with gutta-percha or resilon root canal filling material. *J Endod* **32**(9), 879-81.

- Pitt Ford TR (1979) The leakage of root fillings using glass ionomer cement and other materials. *Br Dent J* **146**(9), 273-8.
- Prado M, Gusman H, Gomes BP, Simao RA (2011) The importance of final rinse after disinfection of gutta-percha and resilon cones. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **111**(6), e21-4.
- Prosser HJ, Wilson AD (1982) Zinc oxide eugenol cements. Vi. Effect of zinc oxide type on the setting reactions. *J Biomed Mater Res* **16**(5), 585-98.
- Qiu Z, Ikehara T, Nishi T (2003) Poly(hydroxybutyrate)/poly(butylene succinate) blends: Miscibility and nonisothermal crystallization. *Polymer* **44**(8), 2503-8.
- Rapisarda E, Bonaccorso A, Tripi TR (1999) [evaluation of two root canal preparation and obturation methods: The mc spadden method and the use of profile-thermafil]. *Minerva Stomatol* **48**(1-2), 29-38.
- Rasal RM, Janorkar AV, Hirt DE (2010) Poly(lactic acid) modifications. *Progress in Polymer Science* **35**(3), 338-56.
- Rathbone S, Furrer P, Lubben J, Zinn M, Cartmell S (2010) Biocompatibility of polyhydroxyalkanoate as a potential material for ligament and tendon scaffold material. *J Biomed Mater Res A* **93**(4), 1391-403.
- Ray HA, Trope M (1995) Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. *Int Endod J* **28**(1), 12-8.
- Reeh ES, Douglas WH, Messer HH (1989) Stiffness of endodontically-treated teeth related to restoration technique. *J Dent Res* **68**(11), 1540-4.
- Reyes-Carmona JF, Felipe MS, Felipe WT (2009) Biomineralization ability and interaction of mineral trioxide aggregate and white portland cement with dentin in a phosphate-containing fluid. *J Endod* **35**(5), 731-6.
- Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR (2006) Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials* **27**(18), 3413-31.
- Ribeiro FC, Souza-Gabriel AE, Marchesan MA, Alfredo E, Silva-Sousa YT (2008) Influence of different endodontic filling materials on root fracture susceptibility. *J Dent* **36**(1), 69-73.
- Ricucci D, Siqueira JF, Jr. (2010) Fate of the tissue in lateral canals and apical ramifications in response to pathologic conditions and treatment procedures. *J Endod* **36**(1), 1-15.
- Roberts HW, Toth JM, Berzins DW, Charlton DG (2008) Mineral trioxide aggregate material use in endodontic treatment: A review of the literature. *Dental Materials* **24**(2), 149-64.

- Robinson MJ, McDonald NJ, Mullally PJ (2004) Apical extrusion of thermoplasticized obturating material in canals instrumented with profile 0.06 or profile gt. *J Endod* **30**(6), 418-21.
- Sagsen B, Er O, Kahraman Y, Orucoglu H (2006) Evaluation of micro leakage of roots filled with different techniques with a computerized fluid filtration technique. *J Endod* **32**(12), 1168-70.
- Sahebi S, Nabavizadeh M, Dolatkhan V, Jamshidi D (2012) Short term effect of calcium hydroxide, mineral trioxide aggregate and calcium-enriched mixture cement on the strength of bovine root dentin. *Iran Endod J* **7**(2), 68-73.
- Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D (2004) Survival of enterococcus faecalis in infected dentinal tubules after root canal filling with different root canal sealers in vitro. *Int Endod J* **37**(3), 193-8.
- Salman SA, Kuroda K, Okido M (2013) Preparation and characterization of hydroxyapatite coating on az31 mg alloy for implant applications. *Bioinorg Chem Appl* **2013**, 175756.
- Santhoskumar A, Palanivelu K, Sharma S, Nayak S (2010) A new synthesis of nickel 12-hydroxy oleate formulation to improve polyolefin's degradation. *Journal of Bioremediation & Biodegradation* **1**(2), 1-9.
- Santos J, Tjaderhane L, Ferraz C (2010) Long-term sealing ability of resin-based root canal fillings. *Int Endod J* **43**, 455-60.
- Santos MD, Walker WA, 3rd, Carnes DL, Jr. (1999) Evaluation of apical seal in straight canals after obturation using the lightspeed sectional method. *J Endod* **25**(9), 609-12.
- Saunders WP (2008) A prospective clinical study of periradicular surgery using mineral trioxide aggregate as a root-end filling. *J Endod* **34**(6), 660-5.
- Sauro S, Mannocci F, Piemontese M, Mongiorgi R (2008) In situ enamel morphology evaluation after acidic soft drink consumption: Protection factor of contemporary toothpaste. *International Journal of Dental Hygiene* **6**(3), 188-92.
- Sawyer AN, Nikonov SY, Pancio AK et al. (2012) Effects of calcium silicate-based materials on the flexural properties of dentin. *J Endod* **38**(5), 680-3.
- Schakenraad JM, Oosterbaan JA, Nieuwenhuis P et al. (1988) Biodegradable hollow fibres for the controlled release of drugs. *Biomaterials* **9**(1), 116-20.
- Scherrer SS, Cesar PF, Swain MV (2010) Direct comparison of the bond strength results of the different test methods: A critical literature review. *Dent Mater* **26**(2), e78-93.
- Schilder H (1974) Cleaning and shaping the root canal. *Dent Clin North Am* **18**(2), 269-96.

- Schilder H, Goodman A, Aldrich W (1974) The thermomechanical properties of gutta-percha. 3. Determination of phase transition temperatures for gutta-percha. *Oral Surg Oral Med Oral Pathol* **38**(1), 109-14.
- Schnieders J, Gbureck U, Thull R, Kissel T (2006) Controlled release of gentamicin from calcium phosphate-poly(lactic acid-co-glycolic acid) composite bone cement. *Biomaterials* **27**(23), 4239-49.
- Shanahan DJ, Duncan HF (2011) Root canal filling using resilon: A review. *Br Dent J* **211**(2), 81-8.
- Shemesh H, Wu MK, Wesselink PR (2006) Leakage along apical root fillings with and without smear layer using two different leakage models: A two-month longitudinal ex vivo study. *Int Endod J* **39**(12), 968-76.
- Shen Y, Haapasalo M (2008) Three-dimensional analysis of cutting behavior of nickel-titanium rotary instruments by microcomputed tomography. *J Endod* **34**(5), 606-10.
- Shipper G, Ørstavik D, Teixeira FB, Trope M (2004) An evaluation of microbial leakage in roots filled with a thermoplastic synthetic polymer-based root canal filling material (resilon). *J Endod* **30**(5), 342-7.
- Shipper G, Teixeira FB, Arnold RR, Trope M (2005) Periapical inflammation after coronal microbial inoculation of dog roots filled with gutta-percha or resilon. *J Endod* **31**(2), 91-6.
- Shipper G, Trope M (2004) In vitro microbial leakage of endodontically treated teeth using new and standard obturation techniques. *J Endod* **30**, 154-8.
- Shuping GB, Ørstavik D, Sigurdsson A, Trope M (2000) Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. *J Endod* **26**(12), 751-5.
- Silveira FF, Soares JA, Nunes E, Mordente VL (2007) Negative influence of continuous wave technique on apical sealing of the root canal system with resilon. *J Oral Sci* **49**(2), 121-8.
- Siqueira JF, Jr., Araujo MC, Garcia PF, Fraga RC, Dantas CJ (1997) Histological evaluation of the effectiveness of five instrumentation techniques for cleaning the apical third of root canals. *J Endod* **23**(8), 499-502.
- Siqueira Jr JF, Rocas IN (2011) Microbiology of primary periapical periodontitis. In: Patel S, Duncan HF, eds. *Pitt ford's problem-based learning in endodontology*. West Sussex, UK: Wiley-Blackwell.
- Sjogren U, Figdor D, Persson S, Sundqvist G (1997) Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *Int Endod J* **30**(5), 297-306.

- Skinner RL, Himel VT (1987) The sealing ability of injection-molded thermoplasticized gutta-percha with and without the use of sealers. *J Endod* **13**(7), 315-7.
- Skrtic D, Antonucci JM (2011) Bioactive polymeric composites for tooth mineral regeneration: Physicochemical and cellular aspects. *J Funct Biomater* **2**(3), 271-307.
- Sly MM, Moore BK, Platt JA, Brown CE (2007) Push-out bond strength of a new endodontic obturation system (resilon/epiphany). *J Endod* **33**(2), 160-2.
- Spangberg L (1982) Endodontic filling materials. In: Smith D, Williams D, eds. *Biocompatibility of dental materials*, pp. 223–57. Boca Raton: CRC Press.
- Spangberg LS, Barbosa SV, Lavigne GD (1993) Ah 26 releases formaldehyde. *J Endod* **19**(12), 596-8.
- Stabholz A, Friedman S, Heling I, Sela MN (1987) Efficiency of different chemical agents in decontamination of gutta-percha cones. *Int Endod J* **20**(5), 211-6.
- Steflik DE, Sisk AL, Parr GR et al. (1993) Osteogenesis at the dental implant interface: High-voltage electron microscopic and conventional transmission electron microscopic observations. *J Biomed Mater Res* **27**(6), 791-800.
- Stein KE, Manfra Marretta S, Siegel A, Vitoux J (2004) Comparison of hand-instrumented, heated gutta-percha and engine-driven, cold gutta-percha endodontic techniques. *J Vet Dent* **21**(3), 136-45.
- Stratton RK, Apicella MJ, Mines P (2006) A fluid filtration comparison of gutta-percha versus resilon, a new soft resin endodontic obturation system. *J Endod* **32**(7), 642-5.
- Stuart CH, Schwartz SA, Beeson TJ (2006) Reinforcement of immature roots with a new resin filling material in a model of apexification. *J Endod* **32**, 350-3.
- Stuart KG, Miller CH, Brown CE, Jr., Newton CW (1991) The comparative antimicrobial effect of calcium hydroxide. *Oral Surg Oral Med Oral Pathol* **72**(1), 101-4.
- Supova M (2009) Problem of hydroxyapatite dispersion in polymer matrices: A review. *J Mater Sci Mater Med* **20**(6), 1201-13.
- Suresh B, Maruthamuthu S, Kannan M, Chandramohan A (2011) Mechanical and surface properties of low-density polyethylene film modified by photo-oxidation. *Polym J* **43**(4), 398-406.
- Swanson K, Madison S (1987) An evaluation of coronal microleakage in endodontically treated teeth. Part 1. Time periods. *Journal of Endodontics* **13**(2), 56-9.
- Tagger M, Greenberg B, Sela G (2003) Interaction between sealers and gutta-percha cones. *J Endod* **29**(12), 835-7.
- Tanner KE (2010) Bioactive ceramic-reinforced composites for bone augmentation. *J R Soc Interface* **7** Suppl 5, S541-57.

- Tay FR, Loushine RJ, Weller RN (2005a) Ultrastructural evaluation of the apical seal in roots filled with a polycaprolactone-based root canal filling material. *J Endod* **31(7)**, 514-9.
- Tay FR, Pashley DH (2007) Monoblocks in root canals: A hypothetical or a tangible goal. *J Endod* **33(4)**, 391-8.
- Tay FR, Pashley DH, Williams MC (2005b) Susceptibility of a polycaprolactone-based root canal filling material to degradation. I. Alkaline hydrolysis. *J Endod* **31(8)**, 593-8.
- Tay FR, Pashley DH, Yiu CK et al. (2005c) Susceptibility of a polycaprolactone-based root canal filling material to degradation. II. Gravimetric evaluation of enzymatic hydrolysis. *J Endod* **31(10)**, 737-41.
- Tay KC, Loushine BA, Oxford C et al. (2007) In vitro evaluation of a ceramicrete-based root-end filling material. *J Endod* **33(12)**, 1438-43.
- Teixeira FB, Teixeira ECN, Thompson JY, Trope M (2004) Fracture resistance of roots endodontically treated with a new resin filling material. *Journal of the American Dental Association* **135(5)**, 646-52.
- Tjaderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T (1998) The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. *J Dent Res* **77(8)**, 1622-9.
- Tokiwa Y, Ugwu CU (2007) Biotechnological production of (r)-3-hydroxybutyric acid monomer. *J Biotechnol* **132(3)**, 264-72.
- Torabinejad M, Eby WC, Naidorf IJ (1985) Inflammatory and immunological aspects of the pathogenesis of human periapical lesions. *J Endod* **11(11)**, 479-88.
- Torabinejad M, Handysides R, Khademi AA, Bakland LK (2002) Clinical implications of the smear layer in endodontics: A review. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontology* **94(6)**, 658-66.
- Torabinejad M, Hong CU, McDonald F, Pitt Ford TR (1995) Physical and chemical properties of a new root-end filling material. *J Endod* **21(7)**, 349-53.
- Torabinejad M, Ung B, Kettering J (1990) In vitro bacterial penetration of coronally unsealed endodontically treated teeth. *J Endod* **16(12)**, 566-9.
- Tormala P, Vasenius J, Vainionpaa S, Laiho J, Pohjonen T, Rokkanen P (1991) Ultra-high-strength absorbable self-reinforced polyglycolide (sr-pga) composite rods for internal fixation of bone fractures: In vitro and in vivo study. *J Biomed Mater Res* **25(1)**, 1-22.
- Tronstad L, Asbjornsen K, Doving L, Pedersen I, Eriksen HM (2000) Influence of coronal restorations on the periapical health of endodontically treated teeth. *Endod Dent Traumatol* **16(5)**, 218-21.

- Trope M, Chow E, Nissan R (1995a) In vitro endotoxin penetration of coronally unsealed endodontically treated teeth. *Endod Dent Traumatol* **11**(2), 90-4.
- Trope M, Maltz DO, Tronstad L (1985) Resistance to fracture of restored endodontically treated teeth. *Endod Dent Traumatol* **1**(3), 108-11.
- Trope M, Moshonov J, Nissan R, Buxt P, Yesilsoy C (1995b) Short vs. Long-term calcium hydroxide treatment of established inflammatory root resorption in replanted dog teeth. *Endod Dent Traumatol* **11**(3), 124-8.
- Tunga U, Bodrumlu E (2006) Assessment of the sealing ability of a new root canal obturation material. *J Endod* **32**(9), 876-8.
- Ulbricht RD, Marretta SM, Baker GJ, Siegel AM, Klippert LS (2002) Comparison of two heated gutta-percha obturation techniques of the mandibular first molar in the dog. *J Vet Dent* **19**(2), 63-70.
- Ungor M, Onay EO, Orucoglu H (2006) Push-out bond strengths: The epiphany-resilon endodontic obturation system compared with different pairings of epiphany, resilon, ah plus and gutta-percha. *Int Endod J* **39**(8), 643-7.
- Ural E, Kesenci K, Fambri L, Migliaresi C, Piskin E (2000) Poly(d,l-lactide/epsilon-caprolactone)/hydroxyapatite composites. *Biomaterials* **21**(21), 2147-54.
- Urayama H, Moon S-I, Kimura Y (2003) Microstructure and thermal properties of polylactides with different l- and d-unit sequences: Importance of the helical nature of the l-sequenced segments. *Macromolecular Materials and Engineering* **288**(2), 137-43.
- Vallet-Regi M, Romero AM, Ragel CV, LeGeros RZ (1999) Xrd, sem-eds, and ftir studies of in vitro growth of an apatite-like layer on sol-gel glasses. *J Biomed Mater Res* **44**(4), 416-21.
- Vallittu PK (1998) Some aspects of the tensile strength of unidirectional glass fibre-polymethyl methacrylate composite used in dentures. *J Oral Rehabil* **25**(2), 100-5.
- van der Meer S, Wijn J, Wolke J (1996) The influence of basic filler materials on the degradation of amorphous d- and l-lactide copolymer. *Journal of Materials Science: Materials in Medicine* **7**(6), 359-61.
- van der Sluis LW, Wu MK, Wesselink PR (2005) An evaluation of the quality of root fillings in mandibular incisors and maxillary and mandibular canines using different methodologies. *J Dent* **33**(8), 683-8.
- Van Ende A, Mine A, De Munck J, Poitevin A, Van Meerbeek B (2012) Bonding of low-shrinking composites in high c-factor cavities. *J Dent* **40**(4), 295-303.
- Verissimo DM, do Vale MS, Monteiro AJ (2007) Comparison of apical leakage between canals filled with gutta-percha/ah-plus and the resilon/epiphany system, when submitted to two filling techniques. *J Endod* **33**(3), 291-4.

- Vertucci FJ (2005) Root canal morphology and its relationship to endodontic procedures. *Endodontic Topics* **10**(1), 3-29.
- Vlachopoulos J, Wagner J (2001) 'The spe guide on extrusion technology and troubleshooting'. Brookfield CT USA: Society of Plastics Engineers.
- Walton R, Torabinejad M (2002) Principles and practice of endodontics. third edn. Philadelphia: Saunders.
- Wang M, Bonfield W (2001) Chemically coupled hydroxyapatite-polyethylene composites: Structure and properties. *Biomaterials* **22**(11), 1311-20.
- Wang M, Hench LL, Bonfield W (1998) Bioglass®/high density polyethylene composite for soft tissue applications: Preparation and evaluation. *Journal of Biomedical Materials Research* **42**(4), 577-86.
- Wang YW, Wu Q, Chen J, Chen GQ (2005) Evaluation of three-dimensional scaffolds made of blends of hydroxyapatite and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) for bone reconstruction. *Biomaterials* **26**(8), 899-904.
- Warneke S, Arenskotter M, Tenberge KB, Steinbuchel A (2007) Bacterial degradation of poly(trans-1,4-isoprene) (gutta percha). *Microbiology* **153**(Pt 2), 347-56.
- Weine F (1996) "Endodontic therapy", 5th edn: St Louis: Mosby
- Weller RN, Jurcak JJ, Donley DL, Kulild JC (1991) A new model system for measuring intracanal temperatures. *J Endod* **17**(10), 491-4.
- Weller RN, Kimbrough WF, Anderson RW (1997) A comparison of thermoplastic obturation techniques: Adaptation to the canal walls. *J Endod* **23**(11), 703-6.
- Wesselink PR (2003) Root filling techniques. In: Bergenholtz g , horsted-bindslev p , reit c. Text book of endodontology. First Edn edn; pp. 286-99. London: Wiley-Blackwell.
- West J (2008) Endodontic predictability--"restore or remove: How do i choose?". In: Cohen M, ed. *Interdisciplinary treatment planning: Principles, design, implementation*, pp. 123-64: Quintessence Publishing Co.
- White JD, Lacefield WR, Chavers LS, Eleazer PD (2002) The effect of three commonly used endodontic materials on the strength and hardness of root dentin. *J Endod* **28**(12), 828-30.
- Whiteley KS, Heggs TG, Koch H, Mawer RL, Immel W (2000) Polyolefins. *Ullmann's encyclopedia of industrial chemistry*: Wiley-VCH Verlag GmbH & Co. KGaA.
- Williams C, Loushine RJ, Weller RN, Pashley DH, Tay FR (2006) A comparison of cohesive strength and stiffness of resilon and gutta-percha. *J Endod* **32**(6), 553-5.
- Williams SF, Martin DP, Horowitz DM, Peoples OP (1999) Pha applications: Addressing the price performance issue: I. Tissue engineering. *Int J Biol Macromol* **25**(1-3), 111-21.

- Work W, Horie K, Hess M, Stepto R (2004) Definition of terms related to polymer blends, composites, and multiphase polymeric materials (iupac recommendations 2004). *Pure and Applied Chemistry* **76**(11), 1985-2007.
- Wright DD, Lautenschlager EP, Gilbert JL (1998) Interfacial properties of self-reinforced composite poly(methyl methacrylate). *J Biomed Mater Res* **43**(2), 153-61.
- Wu MK, Ozok AR, Wesselink PR (2000) Sealer distribution in root canals obturated by three techniques. *Int Endod J* **33**(4), 340-5.
- Wu MK, Tigos E, Wesselink PR (2002) An 18-month longitudinal study on a new silicon-based sealer, rsa roekoseal: A leakage study in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **94**(4), 499-502.
- Wu MK, van der Sluis LW, Wesselink PR (2004) Comparison of mandibular premolars and canines with respect to their resistance to vertical root fracture. *J Dent* **32**(4), 265-8.
- Wu MK, Wesselink PR (1993) Endodontic leakage studies reconsidered. Part i. Methodology, application and relevance. *Int Endod J* **26**(1), 37-43.
- www.Xiameter.com (2009) A guide to silane solutions: Mineral and filler treatment. [15 November 2012].
- Xavier S, Schultz J, Friedrich K (1990) Fracture propagation in particulate-filled polypropylene composites. *Journal of Materials Science Letters* **25**(5), 2428-32.
- Yildirim G, Dalci K (2006) Treatment of lateral root perforation with mineral trioxide aggregate: A case report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **102**(5), e55-8.
- Zandbiglari T, Davids H, Schafer E (2006) Influence of instrument taper on the resistance to fracture of endodontically treated roots. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **101**(1), 126-31.
- Zehnder M, Schmidlin P, Sener B, Waltimo T (2005) Chelation in root canal therapy reconsidered. *J Endod* **31**(11), 817-20.
- Zehnder M, Soderling E, Salonen J, Waltimo T (2004) Preliminary evaluation of bioactive glass s53p4 as an endodontic medication in vitro. *J Endod* **30**(4), 220-4.
- Zhang H, Shen Y, Ruse ND, Haapasalo M (2009a) Antibacterial activity of endodontic sealers by modified contact test against enterococcus faecalis. *J Endod* **35**(7), 1051-5.
- Zhang M, Thomas NL (2011) Blending polylactic acid with polyhydroxybutyrate: The effect on thermal, mechanical, and biodegradation properties. *Advances in Polymer Technology* **30**(2), 67-79.
- Zhang W, Li Z, Peng B (2009b) Assessment of a new root canal sealer's apical sealing ability. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **107**(6), e79-82.

- Zhang Y, Hao L, Savalani MM, Harris RA, Di Silvio L, Tanner KE (2009c) In vitro biocompatibility of hydroxyapatite-reinforced polymeric composites manufactured by selective laser sintering. *J Biomed Mater Res A* **91**(4), 1018-27.
- Zhang Y, Tanner KE, Gurav N, Di Silvio L (2007) In vitro osteoblastic response to 30 vol% hydroxyapatite-polyethylene composite. *J Biomed Mater Res A* **81**(2), 409-17.
- Zidan O, ElDeeb ME (1985) The use of a dentinal bonding agent as a root canal sealer. *J Endod* **11**(4), 176-8.
- Zogheib C, Naaman A, Medioni E, Bourbouze G, Chirani RA (2012) The quality of thermafil obturations with different final apical tapers: A three-dimensional microcomputed tomographic comparative study. *J Contemp Dent Pract* **13**(3), 322-6.
- Zogheib C, Naaman A, Sigurdsson A, Medioni E, Bourbouze G, Arbab-Chirani R (2013) Comparative micro-computed tomographic evaluation of two carrier-based obturation systems. *Clin Oral Investig* **17**(8), 1879-83.
- Zuolo ML, Imura N, Ferreira MO (1994) Endodontic retreatment of thermafil or lateral condensation obturations in post space prepared teeth. *J Endod* **20**(1), 9-12.

Appendix

List of publications in international peer-reviewed journals

1. Raghad Abdulrazzaq Alhashimi, Francesco Mannocci ,Richard M. Foxton, and Sanjukta Deb (2012) “**Synthesis and Preliminary Evaluation of a Polyolefin-based Core for Carrier-based Root Canal Obturation**”. *Journal of Endodontics*, **38**(7), 983-986.
2. Alhashimi R, Mannocci F, Foxton R, Deb S (2014) “**Fabrication and characterization of polymer composites for endodontic use**”. *International Endodontic Journal* **47**(6), 574-82.
3. Alhashimi RA, Foxton R, Romeed S, Deb S (2014) “**An in vitro assessment of gutta-percha coating of new carrier-based root canal obturations**”. *The Scientific World Journal* **2014**, 6.

List of abstracts submitted to national and international conferences

1. Raghad Alhashimi, Sanjukta Deb, Richard Foxton, Francesco Mannocci (oral presentation), “**Design and fabrication of novel root canal obturation materials**” Postgraduate Research Day at King’s College London Dental Institute (2012)
2. Raghad Alhashimi, Sanjukta Deb, Richard Foxton, Francesco Mannocci (oral Presentation), “**Fabrication and efficacy of Polyethylene–Hydroxyapatite composites for endodontic use**” Annual Postgraduate Research Day at King’s College London Dental Institute (2013).
3. Raghad Alhashimi, Sanjukta Deb, Richard Foxton, Francesco Mannocci (oral Presentation), “**Fabrication and Characterization of Polyolefin-Based Composites as Root obturation materials**”. PER/IADR Congress in Helsinki, Finland, September (2012).
4. Raghad Alhashimi, Sanjukta Deb, Richard Foxton, Francesco Mannocci. (Poster Presentation), “**Synthesis and preliminary evaluation of a retrievable polyolefin based core for carrier based root canal obturation**”. United Kingdom Society for Biomaterial Conference, Nottingham (2012).

5. R.A. Alhashimi, F. Mannocci, R.M. Foxton, S. Deb, "***In-vitro comparative assessments of an experimental polyolefin-based core and two commercial cores for carrier-based root canal obturations***" British endodontic society spring scientific meeting (2013), London, UK. [Abstract]. *International Endodontic Journal* **46**(12), 1191-4.
6. Raghad Alhashimi, Sanjukta Deb, Richard Foxton, Francesco Mannocci, (Poster Presentation), "***Fabrication and efficacy of polyethylene-Hydroxyapatite composites for endodontic use***" ESE Biennial Congress, Lisbon, Portugal (2013). [Abstract]. *International Endodontic Journal* 2014; **47**(1), 1-113.
7. Raghad Abdulrazzaq Alhashimi, Francesco Mannocci, Richard M. Foxton, and Sanjukta Deb. (Oral presentation) "**Fabrication and characterisation of polymer composites for endodontic use**". Iraqi division of IADR conference, London, UK (2013): **won a prize of the best presentation**
8. Raghad Abdulrazzaq Alhashimi, Francesco Mannocci, Richard M. Foxton, and Sanjukta Deb. (Poster presentation) "**Fabrication and characterisation of polymer composites for endodontic application**". European Dental Materials Conference, Birmingham, UK (2013).
9. R.A. Alhashimi, F. Mannocci, R.M. Foxton, S. Deb. (Poster presentation) "***In vitro evaluation of an experimental carrier-based root canal obturation system***" British Endodontic Society spring scientific meeting (March 2014)
10. R. AlHashimi, S. Romeed, R. Foxton, S. Deb. (Poster presentation) "***In Vitro Comparative Assessments of an Experimental Polyolefin-Based Core and Two Commercial Cores for Carrier-Based Root Canal Obturations***" American Association of Endodontists annual meeting (30Apr-3May 2014); published in *Journal of endodontics*, Volume 40, Issue 3, March 2014, Pages e1-e47

Scientific awards

1. Best oral presentation prize of Iraqi division scientific meeting of International Association for Dental Research (IADR), London, UK August 2013.
2. Academic excellence award from the Iraqi minister of higher education and scientific research and Iraqi cultural attaché in London (March 2014).

Synthesis and Preliminary Evaluation of a Polyolefin-based Core for Carrier-based Root Canal Obturation

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Abstract

Introduction: Carrier-based gutta-percha is an effective method of root canal obturation creating a 3-dimensional filling; however, retrieval of the plastic carrier is relatively difficult, particularly with smaller sizes. The purpose of this study was to develop composite carriers consisting of polyethylene (PE), hydroxyapatite (HA), and strontium oxide (SrO) for carrier-based root canal obturation. **Methods:** Composite fibers of HA, PE, and SrO were fabricated in the shape of a carrier for delivering gutta-percha (GP) using a melt-extrusion process. The fibers were characterized using infrared spectroscopy and the thermal properties determined using differential scanning calorimetry. The elastic modulus and tensile strength tests were determined using a universal testing machine. The radiographic appearance was established using digital periapical radiographs. **Results:** The composite core carrier exhibited a melting point of 111°C to 112°C, which would facilitate removal by heat application. The elastic modulus and the tensile strength were found to be lower than those of Thermafil carriers (Dentsply Tulsa Dental, Tulsa, OK). The preliminary radiographic evaluation showed that the novel composite core carrier is sufficiently radiopaque and can be distinguished from gutta-percha. **Conclusions:** The PE-HA-SrO composites were successfully melt processed into composite core carriers for delivering gutta-percha into the root canal space. (*J Endod* 2012;38:983–986)

Key Words

Core carrier, hydroxyapatite, melt-extrusion, obturation, polyolefin

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Carrier-based obturation of root canals was first described by Ben Johnson in 1978 (1), and Thermafil (Dentsply Tulsa Dental, Tulsa, OK) was introduced as a carrier-based obturation material with a solid core during the 1990s. Obturators have been designed to correspond to the ISO standardized file sizes and to the tapered nickel-titanium rotary files. The plastic carriers of current Thermafil obturators are made of different materials depending on their size. Smaller sizes (up to size #40) use Vectra (Ticona GmbH, Sulzbach, Germany), which is a liquid crystal polymer insoluble in solvents, and larger sizes use polysulfone, which is soluble in chloroform (2).

Carrier-based obturation systems include Thermafil, Dens-Fil (Dentsply Maillefer, Tulsa, OK), Soft-Core (Axis Dental, Coppell, TX), and resin-based obturators (RealSeal One; SybronEndo, Orange, CA). Common problems reported by clinicians using core-based obturation systems include the lack of apical control (3), the inability to fill oval-shaped canals (4), and the difficult removal of the carrier in case of retreatment (5–8).

Contemporary carriers include grooves to facilitate the removal of the carriers; however, retrievability remains a problem, and the most recently introduced obturators such as the cross-linked gutta-percha (GP) obturators (Gutta-Core, Dentsply Tulsa Dental) have been designed to facilitate the removal by simply trephining through the core. A composite material (30% polypropylene with 70% barium sulfate [Flex Point NEO; Neo Dental Chemical Products, Tokyo, Japan]) has also been proposed as a potential carrier material (9) with a temperature tolerance of about 148°C. However, the material has a high component of barium sulfate as a radiopacifier, which does not assist in the stiffening of the polymer matrix and, thus, acts only as a filler to impart radiopacity.

Hydroxyapatite (HA)-reinforced polyethylene (PE) composites have been successfully used as bone analog materials (10–12) and are currently used as middle ear implants (13). HA/PE composites can be rendered flexible, can be tailored to exhibit desirable flow properties (14–16), and are biocompatible (17–20); thus, they can be excellent candidates for designing carriers for carrier-based obturation systems. HA is used in conjunction with strontium oxide (SrO) to achieve adequate radiopacity as required for a carrier material, and the quantity of the ceramic filler assists in the ability to tailor the flexibility of the carriers. The objective of the present work was to assess the thermomechanical properties of newly designed carriers developed from a low-density PE and HA composite with the inclusion of strontium oxide and to make a preliminary evaluation of their radiopacity.

Materials and Methods

PE with a density of 0.917 g/cm³ (Good Fellow Chemical Products, Huntingdon, UK), HA powders with a mean particle size of 3 to 5 μm (Plasma Biotol, Derbyshire, UK), SrO (Alfa Aesar, Word Hill, UK), and a silane coupling agent A-174 (Merck KGaA, Frankfurt, Germany) were used. Two filler concentrations of 20% and 40% by weight of HA were used to fabricate the composites (LDPEHASR20 and LDPEHASR40). The fibers were extruded using a single screw extruder through a die (Rondol Technology, Staffordshire, UK).

Silanation of HA Filler

HA particles were treated with the A-174 silane coupling agent using a procedure described by Deb et al (11) in 1996. In brief, the silane coupling agent, A-174, was

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dissolved in a 70/30 acetone/water mixture. HA particles were added to the resultant solution and stirred with a magnetic stirrer at room temperature for 1 hour. The temperature was gradually raised while stirring to 100°C and maintained for 1 hour to obtain a slurry. Thereafter, the HA slurry was placed in an oven and maintained at 125°C for 2 hours. The coated HA was washed with water, centrifuged, and dried at 37°C temperature.

Fabrication of HA/PE Composites as Core Carriers for GP

PE and silanated HA with SrO were weighed and prepared by mixing the constituents thoroughly. The mixture was then placed in the hopper of the 12-mm single screw extruder (Rondol Technology). The processing temperature was maintained at 160°C. The speed of the rotary screw was optimized at around 25 rpm with continuous feeding to minimize the risk of polymer thermal degradation. Two different feed compositions were used to fabricate the fibers: the compositions of LDPESHASR20 and LDPESHASR40 were 20% HA, 10% SrO, and 70% low-density PE and 40% HA, 10% SrO, and 50% low-density PE, respectively. The materials were extruded through a die drawn down to different diameters and tapers (Fig. 1A and B).

Subsequently, the composite fibers were successfully coated with GP to form the carrier-based root canal obturator. To coat the core carrier consisting of the experimental fibers, the alpha phase of GP was dissolved in chloroform until a viscous liquid was obtained. A cylindrical rubber tube with a stopper (16-mm length \times different thicknesses) was prepared to receive the composite fiber. GP was then injected using a very fine needle into the tube, creating an evenly distributed layer of GP around the carrier (Fig. 1C). A Soft-Core obturator heater (Axis Dental) was used to heat the experimental HA/PE endodontic obturator.

Characterization of HA/PE Composites as Core Carriers for GP

Five dog bone-shaped specimens were fabricated for the composite carrier and commercially available plastic carrier of Thermafil by melting the materials in a standardized aluminum mold (gauge length = 7.5 mm, width = 2.25, and depth = 1.85 mm) at 150°C and 350°C, respectively. The elastic modulus and tensile strength of the composites were determined by using a Universal Testing Machine (Model 5569 A; Instron, High Wycombe, UK) with a crosshead speed of 5 mm/min as specified by American Society for Testing and Materials (ASTM) Standard D-412-98a for rubber materials (21). Infrared spectroscopy (attenuated total reflectance accessory [Spectrum One, Perkin

Elmer, Waltham, MA) was used to characterize the chemical composition of the experimental materials. The thermal properties of the composite carrier were measured using differential scanning calorimetry (DSC) (Perkin Elmer).

Statistical analysis of the tensile strength and elastic modulus data was performed using GraphPad Prism software version 5 (GraphPad Software Inc, La Jolla, CA). One-way analysis of variance followed by Tukey multiple comparison post hoc tests were used to analyze the data. The level of significance was set at $P = .05$. The radiocontrast of the novel core carrier was initially evaluated both inside and outside the root canal using dental radiography (Planmeca Oy, Helsinki, Finland) in combination with a phosphor plate digital system using the exposure parameters of 60 kV, 7 mA, and 0.2 seconds. In order to evaluate radiopacity, the carriers were heated using the Soft-Core obturator heater and placed into a root canal in a customized mount, which was fabricated to seat a dry mandible. In brief, medium body silicone putty (Aquasil Ultra Heavy Regular Set; Dentsply DeTrey, Konstanz, Germany) was molded to seat the dry mandible. The putty was indented with the mandible to provide a stable seat.

The dry mandible containing the root-filled tooth was placed into a cylinder of acrylic (Plexiglas; Evonik, Essen, Germany) (15 \times 15 \times 1 cm) in order to obtain an attenuation of the beam, which was comparable with the presence of the soft tissues in the clinical situation. The block was fixed in position with 3M Micropore Surgical Tape (3M, St Paul, MN). The X-ray tube head and digital sensor were aligned to allow radiographs to be exposed using the paralleling technique. The microstructure of the fibers was visualized using a stereomicroscope (LS Starrett, Jedburgh, UK).

Results

Physical Properties

The silanation of the HA was confirmed by the FTIR spectra of the silanated and unsilanated HA. The silanated HA exhibited a characteristic peak at 1720 cm^{-1} attributed to the carbonyl group arising from the gamma-methacryloxypropyltrimethoxysilane coupled to the HA, with other peaks at 1637 cm^{-1} , 1460 cm^{-1} , and 1300 cm^{-1} because of the $-\text{C}=\text{C}-$, $\text{C}-\text{C}-$, and $\text{Si}-\text{O}$ stretching frequencies, respectively, as shown in Figure 2A. The FTIR spectra of both LDPESHASR20 and LDPESHASR40 are shown in Figure 2B, which are very similar to each other as expected; however, the intensity of the phosphate peaks vary because of the compositional changes. The 2 sharp peaks at 2848 cm^{-1} and 2916 cm^{-1} are caused by $-\text{CH}_2$ stretching and $-\text{CH}_2$ deformation arising from the polyethylene segments, respectively; absorption

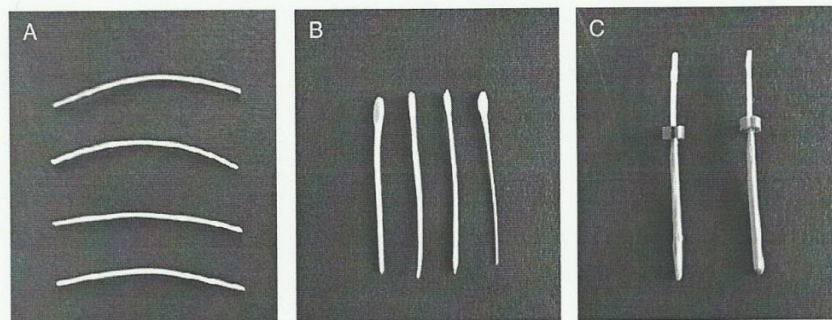


Figure 1. (A) Typical extruded fibers obtained from the melt-extrusion process of LDPESHASR20. (B) Fibers in different diameters and tapers. (C) Novel obturator.

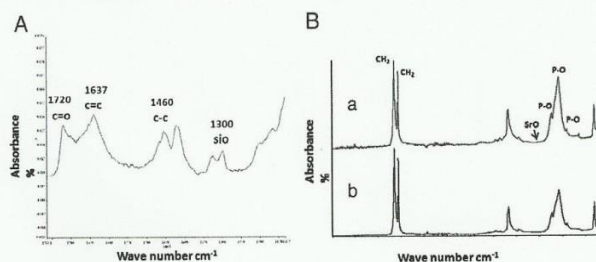


Figure 2. Attenuated total reflectance-FTIR spectra of (A) the silanated HA particles and (B) (a) LDPEHASR40 and (b) LDPEHASR20.

bands at 962 cm^{-1} , 1022 cm^{-1} , and 1091 cm^{-1} are characteristic of the phosphate groups; and P-O is seen in the HA group. The weak peak at 1150 cm^{-1} was assigned to SrO.

The DSC measurements showed that the LDPEHASR20 and LDPEHASR40 composites had a melting temperature range between 111°C and 112°C in contrast to 350°C exhibited by the plastic carrier used for Therafil. The lower temperature indicated that the temperatures required to remove the experimental carriers were more suited to the clinical situation.

The tensile strengths of the PE containing 20% HA and 40% HA composites were found to be $10.89 \pm 0.28\text{ MPa}$ and $10.80 \pm 1.39\text{ MPa}$, respectively, which indicated that there was no significant difference between the HA/PE groups ($P > .05$). However, the plastic carrier of Therafil showed a strength of $36.9 \pm 0.98\text{ MPa}$, which was significantly higher than that of LDPEHASR20 and LDPEHASR40 composite carriers ($P < .001$). The elastic modulus of the Therafil carrier ($10.4 \pm 3.33\text{ GPa}$) was significantly higher ($P < .001$) than that of PE containing 20% HA ($0.61 \pm 0.11\text{ GPa}$) and 40% HA composites ($0.91 \pm 0.09\text{ GPa}$).

Radiographic and Microscopic Assessment

The radiographs showed that the novel core carrier was sufficiently radiopaque and easily distinguishable from GP, particularly when examined inside the dry mandible (Fig. 3A-C). Microscopically, the fibers revealed a homogenous dispersion of the HA filler within the matrix and a slight irregularity of the external surfaces indicating that the fibers can be coated with GP obtaining a good micromechanical adhesion (Fig. 3D).

Discussion

The HA/PE composites were fabricated using silanated hydroxyapatite, which enhances the interfacial bonding between the filler and

matrix, thus enhancing the properties of the composite (11). A single screw extruder was used to fabricate the novel core carrier device. Fibers were drawn and tapers were produced as required using appropriate dies. The concentration of the filler was selected on the basis of earlier studies, which indicated that increasing the filler concentration made processing more difficult and optimum properties could be achieved at about 40% HA. However, for the current application, a balance between the flexibility and rigidity is required; hence, low and high concentrations of HA were used although the maximum percentage of filler was limited to 40%.

The melting point of the composites was around 111°C as determined by DSC, which is in agreement with the observations of a previous study (14). Infrared spectroscopy confirmed the presence of HA and PE the composites with no degradation occurring during the processing. The main bands of PE were observed at 718 cm^{-1} , 1468 cm^{-1} , 2849 cm^{-1} , and 2916 cm^{-1} . This is in agreement with the findings of an earlier study (22). New peaks, particularly at 1033 cm^{-1} and 1758 cm^{-1} , displayed in the spectra were associated with the presence of HA and SrO. These results suggested the presence of a strong intermolecular interaction between the polymer matrix and filler.

The melting point of the composite indicates that the core carrier can be removed easily and safely from the root canal space using a conventional heat source at a temperature of 85°C to 100°C . Such temperatures are far below the threshold of 200°C that is associated with the risk of damage to the periodontal tissues (23). This would not be possible with Therafil plastic carriers, which have a melting point around 350°C . This is particularly relevant for plastic carriers size #40 or smaller because they are not soluble in commonly used solvents such as chloroform (2). Also, the retrieval of the carrier was found to be more difficult for canals of a larger diameter or taper (24). Further studies are needed to compare the retrievability of these

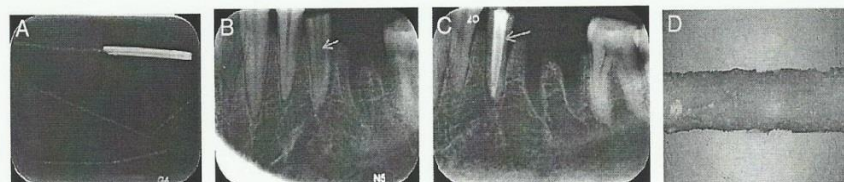


Figure 3. (A) A radiograph showing the newly designed carrier made of LDPEHASR20. (B) A radiograph showing the newly designed carrier made of LDPEHASR20 inserted in the root canal space of an extracted tooth positioned into an alveolus of a dry mandible (arrow). (C) LDPEHASR20 carrier coated with gutta-percha inserted in the root canal space of an extracted tooth positioned into an alveolus of a dry mandible (arrow). (D) The microscopic appearance of the fabricated LDPEHASR20 composite before gutta-percha coating ($2\times$).

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new carrier types with that of the established core-based systems such as Therafil or Gutta-Core.

In order to develop a material with the best possible mechanical properties, composite materials with 2 different concentrations of HA (20% and 40%) were tested. The tensile strength measurements showed that PE containing 20% HA and 40% HA exhibited a tensile strength lower than that of Therafil plastic carriers. There was no significant difference between the composite containing 20% HA and 40% HA. However, the lower tensile strengths of the experimental fibers are high enough to withstand any tensile forces to which the carrier is subjected.

The measurements of the elastic modulus of the experimental composite material revealed that it has a certain degree of flexibility. In particular, the composite containing 20% HA with 10% SrO showed an outstanding flexibility compared with the composite containing 40% HA with 10% SrO. One of the requirements of core materials is an optimum flexibility that will allow the material to follow the curvature of the canals. Although further studies are needed to assess the ability of the experimental core materials to follow the curvatures and to provide a good seal in curved canals, this study indicates that a good clinical handling can be achieved.

In comparison to Flex Point Neo, the current composites are fabricated with a highly biocompatible material. HA and the addition of the ceramic provided the additional potential of varying the physical properties by varying the quantity of the filler.

HA low-density PE composites show good potential as carrier materials, and according to preliminary findings, they also show a good adhesion between GP and the composite. However, further studies are required to assess the interaction between the carrier material and GP along with a systematic radiographic assessment and micro-leakage tests. Studies on the sterilizability of the new core material are in progress; however, because the carrier is covered with GP, the sterilizability of the obturators in autoclave remains problematic. In conclusion, the novel HA/PE composites were found to be suitable as core materials for carrier-based root canal fillings.

Acknowledgments

The authors deny any conflicts of interest related to this study.

References

- Johnson WB. A new gutta-percha technique. *J Endod* 1978;4:184-8.
- Parker H, Glickman GN. Solubility of plastic Therafil carrier. *J Dent Res* 1993;72:188.
- Gutmann JL, Saunders WP, Saunders EM, Nguyen L. An assessment of the plastic Therafil obturation technique: part 1. Radiographic evaluation of adaptation and placement. *Int Endod J* 1993;26:87-92.
- De-Deus G, Reis C, Bezons D, de Abanches AM, Coutinho-Filho T, Paciornik S. Limited ability of three commonly used thermoplasticized gutta-percha techniques in filling oval-shaped canals. *J Endod* 2008;34:1401-5.
- Ibarrola JL, Knowles KL, Ludlow NO. Retrieval of Therafil Plastic cores using organic solvents. *J Endod* 1993;19:417-8.
- Gutmann JL, Dumsha TC, Lovdahl PE. *Problem Solving in Endodontics*, 4th ed. St Louis, MO: Elsevier; 2006. 204-55.
- Zuolo ML, Imura N, Ferreira MO. Endodontic retreatment of Therafil or lateral condensation obturations in post space prepared teeth. *J Endod* 1994;20:9-12.
- Wesselink P. Root filling techniques. In: Bergenholz G, Horsted-Bindslev, Reit C, eds. *Textbook of Endodontology*, 1st ed. London: Wiley-Blackwell; 2003: 286-99.
- Kato H, Nakagawa K. FP core carrier technique: Thermoplasticized gutta-percha root canal obturation technique using polypropylene. *Bull Tokyo Dent Coll* 2010; 51:213-20.
- Bonfield W. Composites for bone replacement. *J Biomed Eng* 1988;10:522-6.
- Deb S, Wang M, Tanner KE, Bonfield W. Hydroxyapatite-polyethylene composites: effect of grafting and surface treatment of hydroxyapatite. *J Mater Sci Mater Med* 1996;7:191-3.
- Wang M, Joseph R, Bonfield W. Hydroxyapatite-polyethylene composites for bone substitution: effects of ceramic particle size and morphology. *Biomaterials* 1998; 19:2357-66.
- Tanner KE. Bioactive ceramic-reinforced composites for bone augmentation. *J R Soc Interface* 2010;7:S541-57.
- Madera-Santana TJ, Robledo D, Azamar JA, Rios-Soberanis, Freile-Pelegrin Y. Preparation and characterization of low density polyethylene-Agar biocomposites: torque-rheological, mechanical, thermal and morphological properties. *Polym Eng Sci* 2010;585-91.
- Suresh B, Maruthamuthu S, Kannan M, Chandramohan A. Mechanical and surface properties of low-density polyethylene film modified by photo-oxidation. *Polym J* 2011;43:398-406.
- Ngu JLS, Akmal NM, Chuah LA, Choong T, Yaw S. Characterization of mechanical properties: low density polyethylene composite using α -alumina submicrometer sized particle as filler. Malaysia Polymer International Conference (MPIC 2009). October 21-22, 2009, Malaysia National University, Bangi, Selangor.
- Zhang Y, Tanner K, Gurav N, Di Silvio L. In vitro osteoblastic response to 30 vol % hydroxyapatite-polyethylene composite. *J Biomed Mater Res A* 2007;81: 409-17.
- Parra C, Gonzalez G, Albano C. Synthesis and characterization of composite materials HDPE/HA and PMMA/HA prepared by sonochemistry. *Macromol Symp* 2009;286:60-9.
- Di Silvio L, Dalby MJ, Bonfield W. Osteoblast behaviour on HA/PE composite surfaces with different HA volumes. *Biomaterials* 2002;23:101-7.
- Bonfield W, Tanner K. Hydroxyapatite composite biomaterials—evolution and applications. *Mater World* 1997;5:18-20.
- ASTM International. *ASTM D412-98a(2002)e1 Standard Test Methods for Vulcanized Rubber and Thermoplastic Elastomers—Tension*. West Conshohocken, PA: ASTM International; 2002.
- Santhoskumar AU, Palanivelu K, Sharma SK, Nayak SK. A new synthesis of nickel 12-hydroxy oleate formulation to improve polyolefin degradation. *J Bioremed Biodegrad* 2010;1:1-9.
- Lipski M, Wozniak K. In vitro Infrared thermographic assessment of root surface temperature rises during Therafil retreatment using system B. *J Endod* 2003; 29:413-5.
- Hayakawa T, Tomita F, Okiji T. Influence of the diameter and the taper of root canals on the removal efficiency of Therafil plus plastic carriers using protaper retreatment files. *J Endod* 2010;36:1676-8.

Fabrication and characterization of polymer composites for endodontic use

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Abstract

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Aim To develop a low-density polyethylene–hydroxyapatite (HA-PE) composite with properties tailored to function as a potential root canal filling material.

Methodology Hydroxyapatite and polyethylene mixed with strontium oxide as a radiopacifier were extruded from a single screw extruder fitted with an appropriate die to form fibres. The composition of the composite was optimized with clinical handling and placement in the canal being the prime consideration. The fibres were characterized using infrared spectroscopy (FTIR), and their thermal properties determined using differential scanning calorimetry (DSC). The tensile strength and elastic modulus of the composite fibres and gutta-percha were compared, dry and after 1 month storage in simulated body fluid (SBF), using a universal testing machine. The radiopacity of the fibres was determined using digital radiography. The interaction of the composites with eugenol was evaluated and compared with gutta-percha. Data of the

tensile test were submitted to two-way ANOVA and Bonferroni tests ($P < 0.05$).

Results The endothermic peaks obtained from the DSC studies showed that the melting point of the HA/PE composites ranged between 110.5 and 111.2 °C, whereas gutta-percha exhibited a melting point at 52 °C. The tensile strength and elastic modulus of the silanated HA/PE composites were significantly higher than those of gutta-percha ($P < 0.0001$) under dry conditions and 1 month storage in SBF. The gutta-percha in eugenol showed a significant increase in the polymer molar mass, whereas the silanated HA/PE composites were unchanged. Radiological evaluations demonstrated that silanated HA/PE fibres were sufficiently radiopaque.

Conclusion Promising materials for endodontic applications have been developed, offering relevant benefits over the traditional materials in terms of mechanical and chemical properties.

Keywords: melt-extrusion, polyethylene–hydroxyapatite, root canal filling material, stiffness, tensile strength.

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Introduction

Many different materials have been advocated as root filling materials; however, with the prevailing standards of filling root canals with gutta-percha and sealers, development of newer materials with properties

better suited for this application has been limited. Ideally, a material used for root filling should be biocompatible (Scotti *et al.* 2008), nonresorbable, impervious to dissolution by tissue fluids, provide a hermetic seal and possess good handling characteristics. Obtaining a filled root canal, free of voids, along with chemomechanical preparation of the root canal is important to achieve long-term success (Ng *et al.* 2011).

Gutta-percha has been widely used as a root canal filling material for almost a century (Gutmann & Witherspoon 2002). Disadvantages of gutta-percha include susceptibility to leakage (Teixeira *et al.* 2004,

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Shipper *et al.* 2005, Tunga & Bodrumlu 2006), ease of displacement under pressure (Nguyen 1994), physicochemical instability (Tagger *et al.* 2003, Chandrasekhar *et al.* 2011) and the inability to sterilize it in autoclaves or use other conventional sterilization techniques (Stabholz *et al.* 1987).

Filling of the root canal system is an integral component in promoting periapical healing and preventing disease progression. The root filling material accomplishes this by reducing microleakage and entombing bacteria remaining in the root canal space. The effectiveness of a material to adequately seal the root canal space is established by its physical properties and handling characteristics. Gutta-percha has been the preferred root canal filling material because it possesses many favourable properties, which include biological compatibility, pliability, easy placement and removal, and radiopacity (Weine 1996). Studies have shown that gutta-percha can be well adapted to the root canal walls using various filling techniques (Collins *et al.* 2006, Natera *et al.* 2011); however, this is successful only in conjunction with a sealer. Despite its close proximity, it has been shown that gutta-percha does not create a complete dentinal seal (Shipper *et al.* 2005). Potential unfilled spaces may allow coronal microleakage and re-infection of the root canal system, which may contribute to treatment failures (Madison *et al.* 1987, Swanson & Madison 1987). To address this problem, advancements in polymer technology have led to the development of resin-based root filling materials. A polymer filling material, Resilon® (Resilon Research LLC, Madison, CT, USA), has been shown to reduce microleakage by effective sealing of the root canal system (Shipper *et al.* 2004). In addition to the aforementioned properties of gutta-percha, Resilon® has been shown to increase the resistance to fracture of root filled teeth and to reduce periapical inflammation after microbial inoculation (Teixeira *et al.* 2004). On the other hand, it has been shown that the polyester component of Resilon® is subject to a slow degradation process in the presence of moisture (Tay *et al.* 2005). Composites are an attractive approach for developing materials where there is need to regulate the mechanical and physical properties. Ideally, a root filling material should be rigid enough to be placed into the canal space and provide both an apical and coronal seal. Root filled teeth are considered to be more susceptible to fracture than teeth with vital pulps because of the reduced amount of tooth structure (Ikram *et al.* 2009); thus, producing a root

filling material with a modulus of elasticity close to that of dentine might help prevent root fractures. The adaptation of the filling material to the root canal walls is also an important requirement, and a simple placement technique is desirable. In this study, the development of a Hydroxyapatite-low-density polyethylene composite as a new root canal filling material is reported. Previous studies have shown that similar composites could be successfully used as bone analogue materials (Bonfield 1988, Deb *et al.* 1996), which exhibit excellent cytocompatibility. Based on the fact that tooth tissue is a composite of hydroxyapatite and collagen, a composite using low-density polyethylene to impart a degree of flexibility and a mineral component similar to that of dentine was selected for the study. The fabrication process of the composite material, its efficacy as a root canal filling material and a comparison with gutta-percha in terms of physical, mechanical and thermal properties are described. The null hypothesis is that HA-low-density polyethylene composites are not suitable as root canal filling materials.

Materials and methods

Polyethylene (PE) with a density of 0.917 g cm^{-3} (Good Fellow Chemical Products, Huntingdon, UK), hydroxyapatite (HA) powders with a mean particle size of $3\text{--}5 \mu\text{m}$ (Plasma Biotol, Tideswell, UK), strontium oxide (SrO) (Alfa Aesar, Word Hill, UK), silane coupling agent A-174, (3 Trimethoxysilyl propyl methacrylate), (Merck, Frankfurt, Germany) and gutta-percha (Cybertech, London, UK) were used. Based on a pilot study, two concentrations of 20% and 30% by weight of hydroxyapatite were selected for extruding the composites. To impart adequate radiopacity, SrO was added. Thus, two sets of composites were developed with 20% HA + 30% SrO and 30% HA + 20% SrO and labelled as LDPEHASR20/30 and LDPEHASR30/20, respectively. A parallel set of composites using nonsilanated HA were also prepared. Composite fibres were extruded using a single screw extruder through a die hole (Rondol Technology, Stoke-on-Trent, UK).

Silanation of hydroxyapatite

Hydroxyapatite particles were treated with A-174 silane coupling agent using a procedure described by Deb *et al.* (1996). In brief, 10% by weight (with respect to hydroxyapatite) of the silane coupling

agent, A-174 was dissolved in a 70/30 acetone/water mixture. Hydroxyapatite particles were added to the resultant liquid and stirred with a magnetic stirrer at room temperature for 1 h and the temperature gradually raised whilst stirring to 100 °C and maintained for 1 h to obtain a slurry. Thereafter, the hydroxyapatite slurry was placed in oven maintained at 125 °C for 2 h until the solvent had evaporated. The coated hydroxyapatite was washed with water, centrifuged and dried at 37 °C temperature.

Fabrication of HA/PE composites

Pre-powders were prepared by thoroughly mixing polyethylene (PE), silanated hydroxyapatite (HA) and strontium oxide (SrO) in proportions as described earlier. The mixture was then placed in the hopper of the single screw extruder (12 mm single screw, Rondol Technology). The processing temperature was maintained at 160 °C. The speed of the rotary screw was optimized at around 25 rpm with continuous feeding to minimize the risk of polymer thermal degradation. Four different feed compositions were used to fabricate the fibres, and the composition is shown in Table 1. The materials were then extruded through a die and drawn down to different diameters and tapers.

Characterization of HA/PE composites

Five dog-bone shaped specimens were fabricated for the four composite fibres and for the commercially available gutta-percha by thermoplasticizing the materials in a standardized aluminium mould (gauge length 7.5 mm, width 2.25, depth 1.85 mm) at 160 and 100 °C, respectively, using a dental oven (Weiss-Gallenkamp, Loughborough, UK). The elastic modulus and tensile strength were determined using a cross-head speed of 5 mm min⁻¹ as specified by the ASTM Standard D-412-98a for rubber materials (ASTM International 2002, Williams *et al.* 2006). The tensile strengths were calculated using stress (force per unit area) - strain (deformation) equation. Infrared

spectroscopy (ATR accessory, Spectrum One, Perkin Elmer, Waltham, MA, USA) was used to characterize the chemical composition of the experimental materials. FTIR spectra were recorded at room temperature in the range from 4000 cm⁻¹ to 650 cm⁻¹. The melting point of the composite carrier was measured using differential scanning calorimetry (DSC) (DSC, Perkin Elmer). The tensile strength of the composites and gutta-percha were determined using a universal testing machine (Instron model 5569 A Series Dual Column, High Wycombe, UK) at 24 h under dry conditions and also after the specimens were stored in simulated body fluid (SBF) for 1 month at 37 °C. In addition, five weighed cones (20 mm-long) of silanated, nonsilanated composite fibres and gutta-percha were immersed in eugenol. The interaction of the composites and gutta-percha with eugenol was assessed by measuring gravimetric (polymer molar mass) changes after 1, 2 and 5 days of immersion in eugenol.

The radiopacity of the HA/PE root filling material was assessed using a dental radiography equipment (Planmeca Oy, Helsinki, Finland) in combination with a phosphor plate digital system and a grey scale value aluminium step wedge, which represents the different shades between black and white varying from 0 to 255 pixels, where 0 represents black and 255 represents white. The grey scale measurement was then converted into mm equivalents of aluminium. The exposure parameters were 70 kV, 8 mA and 0.2 s. The object-to-focus distance was 30 cm. The degree of radiopacity was determined using an aluminium washer, 1 mm thick with an internal diameter of 10 mm, filled with the new material and radiographed together with the graduated aluminium step wedge, with thickness varying from 1 mm to 10 mm in steps of 1 mm each (ANSI/ADA 2000 specification number 57) (ANSI/ADA 2000). The radiopacity of the filling material inside the washer was compared with the steps of the aluminium step wedge on a computer screen using the Digora radiographic software (Soredex Orion Corporation, Helsinki, Finland). Statistical analyses were performed using

Table 1 Composition of the experimental composites

Composite	Composition
LDPESHASR20/30	Low-density polyethylene + 20% silanated hydroxyapatite + 30% strontium oxide
LDPESHASR20/30	Low-density polyethylene + 20% nonsilanated hydroxyapatite + 30% strontium oxide
LDPESHASR30/20	Low-density polyethylene + 30% silanated hydroxyapatite + 20% strontium oxide
LDPESHASR30/20	Low-density polyethylene + 30% nonsilanated hydroxyapatite + 20% strontium oxide

GraphPad Prism software version 5 (La Jolla, CA, USA). Two-way analysis of variance followed by Bonferroni multiple comparison post hoc tests were used to analyse the data of cohesive strength and stiffness. The level of significance was assessed using $\alpha = 0.05$.

Cytocompatibility of HA/PE composite

The cytocompatibility of the HA/PE carrier was assessed using human osteoblast-like cells (HOB obtained at passage number 18, Tc4). The cell culture was performed at 37 °C in a humidified atmosphere with 5% CO₂ in Dulbecco's modified eagles medium (DMEM), as previously described by Camilleri *et al.* (2005). The experimental composites were placed in aluminium washer to produce discs (12 mm in diameter). Two discs of test material were used for the indirect test and three discs for the direct test. The test materials were assessed for the *in vitro* biocompatibility according to ISO 10993-Part 5, 1992. The cytotoxicity of the eluant was evaluated. The test materials were placed into labelled containers filled with Dulbecco's modified eagles medium (DMEM), supplemented with 10% foetal calf serum (FCS), seeded with HOB cells (1×10^4 cells mL⁻¹) and incubated for 1 day to allow cell confluence. The containers were sealed with a parafilm and placed onto a roller mixer (Luckham 4RT, Burgess Hill, UK). Methyltetrazolium (MTT) assay was performed to measure the cell metabolic function; the plates were removed from the incubator, and 100 µL of media was removed from the wells and submitted to elution assessed at 24 and 72 h cell exposure time. Standard culture medium was used as a negative nontoxic control and 10% ethanol diluted in media as a positive control.

Alamar Blue™ cell proliferation assay

At selected time-points 3, 7, 14, 21 and 28 days, 100 µL media was mixed with 1 mL of Alamar Blue™ (diluted 1 : 10 in phenol red-free) and incubated for 4 h at 37 °C, 5% CO₂. Wells without any cells were used as the blank control, whilst Thermanox™ was used as nontoxic negative control. Following the incubation, 8 × 100 µL aliquots from each well were taken and transferred to a 96-well plate. Absorbance was measured on a fluorescent plate reader (emission wavelength of 570 nm; excitation wavelength 540 nm). Statistical analysis was performed using the Pairwise *t*-test with ($P < 0.05$).

Results

Figure 1A shows the infrared spectra of the hydroxyapatite particles after being treated with the silane coupling agent. The presence of absorption bands at 1720 cm⁻¹ and (1300–1200 cm⁻¹) can be assigned to the carboxyl group and SiO group, respectively, indicating the availability of the coupling agent on the surface of hydroxyapatite. The silanated HA exhibited characteristic peaks at 1720, 1637, 1460 and 1300 cm⁻¹ that were attributed to the chemical bands C=O, C=C, C-C and Si-O, respectively, in the HA particles. The FTIR spectra of LDPEHASR20/30 and LDPEHASR20/30 are shown in Fig. 1B, with the two sharp peaks at 2848 and 2916 cm⁻¹ attributed to the -CH₂ symmetric and asymmetric stretching, respectively, with a peak at 1465 cm⁻¹ arising due to the bending deformation of the -CH₂ groups of the polyethylene. The weak peaks at 1370 cm⁻¹ and the strong absorption band between 730 and 710 cm⁻¹ are due to the -CH₃ symmetric deformation and

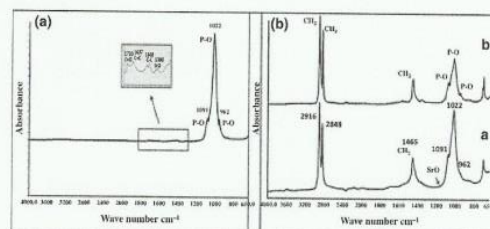


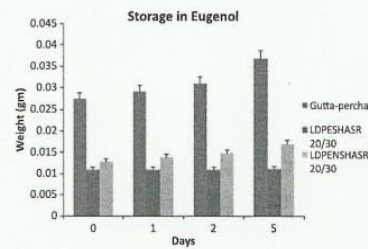
Figure 1 (a) ATR-FTIR spectra of the silanated hydroxyapatite particles and (b) of the (a) LDPEHASR30/20 (b) LDPEHASR20/30.

Table 3 Tensile strength and elastic modulus of the hydroxyapatite/polyethylene/SrO composite fibres and gutta-percha

Composite	Dry		Wet	
	Tensile strength (MPa) Mean \pm SD	Elastic modulus (GPa) Mean \pm SD	Tensile strength (MPa) Mean \pm SD	Elastic modulus (GPa) Mean \pm SD
LDPEHASR20/30 ^a	14.2 (1.5)	1.2 (0.07)	14.9 (0.07)	1.2 (0.30)
LDPEHASR20/30 ^b	11.4 (1.9)	1.0 (0.18)	10.6 (3.10)	0.8 (0.10)
LDPEHASR30/20 ^a	16.3 (1.0)	1.4 (0.10)	19.0 (3.55)	1.4 (0.59)
LDPEHASR30/20 ^b	11.7 (1.4)	1.2 (0.08)	12.8 (1.67)	1.3 (0.01)
Gutta-percha	7.0 (0.8)	0.07 (0.01)	8.0 (0.86)	0.1 (0.01)

^aTwo-way analysis of variance showed significant differences for the elastic modulus ($P > 0.0001$) and tensile strength ($P > 0.001$) of the dry and wet silanated and nonsilanated composites.

^bNon-significant difference ($P < 0.05$) for the tensile strength of the wet nonsilanated composite.

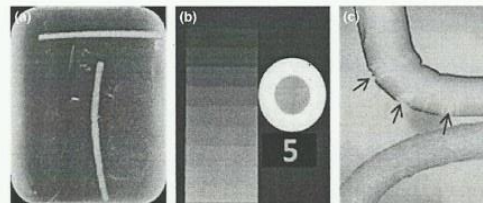
**Figure 2** Polymer mass of the experimental materials and gutta-percha before and after immersion in eugenol.

Unfavourable properties of gutta-percha as a root filling material also include its inability to strengthen a root filled tooth (Teixeira *et al.* 2004) and the inability to be sterilized in autoclave due to the dimensional changes that occur as a result of its low melting temperature. The null hypothesis of the present study is rejected as the experimental composites developed exhibit adequate properties as potential new endodon-

tic materials with high elastic modulus and tensile strength, which can potentially reinforce the endodontically treated root.

Matrix composition, filler type, size and size distribution of filler particles, quality of interfacial bonding and the amount of filler can all affect the physical and mechanical properties of composites (Labella *et al.* 1994). In this study, the filler used was hydroxyapatite. The mean particle size and size distribution were maintained the same, and the selection criteria of the composition were based on a pilot study in relation to extrusion parameters. HA fillers with a size of 3–5 μ m were included in the composite to obtain the best possible distribution of fillers within the polymer matrix, considering the clinical handling properties of the composite. Besides, HA fine particles helped enhancing the radiopacity of the material.

The filler content was limited to 20% and 30% by weight of hydroxyapatite with the addition of strontium oxide to impart radiopacity. The net inorganic content was maintained at 50% by weight in both of the experimental composites with 20% and 30% by weight of SrO. FTIR spectra were recorded to confirm

**Figure 3** (a) Radiograph shows LDPEHASR20/30 composite, (b) the radiopacity of LDPEHASR20/30 composite in the washer is estimated in relation to the thickness of aluminium steps wedge, (c) stereomicroscopic image (1 \times) showing cracks in gutta-percha when bent (arrows) compared with intact LDPEHASR20/30.

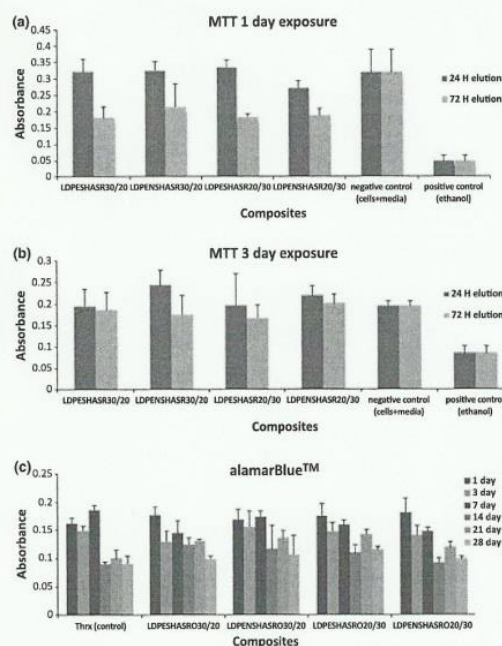


Figure 4 (a) Viability of human osteoblast-like cells (HOB) following exposure for 1 day eluted media from the experimental composites, expressed as mean absorbance \pm SD (b) Viability of HOB cells following exposure for 3 day eluted media from the experimental composites, expressed as mean absorbance \pm SD, and (c) metabolic activity of HOB cells as indicated by Alamar Blue™ assay in direct contact with the experimental composites at 1, 3, 7, 14, 21 and 28 days.

the components of the novel composites. The main bands of LDPE were at 718, 1468, 2849 and 2916 cm^{-1} . This observation is in agreement with the findings of a previous study (Santhoskumar *et al.* 2010). New peaks particularly at 1033 and 1758 cm^{-1} were observed in the infrared spectra of LDPE by adding hydroxyapatite fillers and strontium oxide. This result indicated a strong intermolecular interaction between the polymer matrix and filler. The inclusion of 20% or 30% by weight of strontium oxide in the composites in conjunction with hydroxyapatite imparted adequate radiopacity to the fibres, required for endodontic filling materials. The tensile strength and elastic modulus data proved that the fibres of LDPESHASR20/30 had sufficient rigidity and outstanding flexibility.

The new filling material, in particular LDPE-SHASR20/30 offered considerable benefits over gutta-percha in terms of the mechanical properties. Zinc oxide – eugenol is often used in the root canal, and thus, the interaction of eugenol with the filling material is important. As the reaction of zinc oxide – eugenol occurs over variable periods of time, it is possible that the filling material may come in contact with free eugenol (Prosser & Wilson 1982, Camps *et al.* 2004), and LDPESHASR composites exhibited good stability without undergoing any weight change when immersed in eugenol or SBF in contrast to gutta-percha. Although the experimental study was carried out under aggressive conditions, where the gutta-percha point was placed in neat eugenol, which does not occur in a clinical situation, the distortion

and swelling of the gutta-percha point may cause decomposition of the trans-polyisoprene in the gutta-percha and result in voids within the root canal filling that could be occupied by bacterial biofilms.

The polyethylene-hydroxyapatite composites showed an adequate amount of flexibility required to introduce the endodontic points without breakage. This might be an advantage over gutta-percha especially in canals of small diameters that require the use of thin filling points. Furthermore, the SrO, contained in the new material, may exert an antibacterial action and potentially help in eliminating bacteria from the root canal space (Guida et al. 2003).

The melting point data obtained from DSC measurements showed that the experimental fibres had significantly higher values ($P < 0.005$; Table 2) in comparison with gutta-percha. Literature values reported for the melting temperature of Resilon, a relatively new root filling material, ranged from 60 °C to 70 °C, which is lower than the experimental composites. The enthalpy changes from the DSC measurements which indicate the amounts of heat absorbed and released during the process were significantly higher for the experimental composite in comparison with gutta-percha. The results of the melting point determination for gutta-percha are consistent with other studies (Combe et al. 2001). The higher melting point of the newly developed materials will require the development of new devices suitable for warm compaction techniques.

The radiopacity of LDPESHASR20/30 was superior to LDPESHASR30/20 indicating that the SrO content enhances radiopacity. The physical and mechanical behaviour of LDPESHASR20/30 composite in eugenol and SBF did not change significantly. Conversely, gutta-percha and LDPENSHASR20/30 showed some changes particularly in eugenol due to the ionic reaction between eugenol and the zinc oxide that is included as filler in the gutta-percha points, identifying the importance of the silanation process for the development of a more stable material.

The present study demonstrated that gutta-percha unlike the composite materials expanded after 1, 2 and 5 days of immersion in eugenol. Similarly, the tensile strength and stiffness of silanated HA/PE composites particularly LDPESHASR20/30 composite were superior to those of gutta-percha in both dry and wet conditions. Previous studies also have shown a volumetric expansion of gutta-percha due to contact with eugenol containing sealers (Michaud et al. 2008) and suggested that gutta-percha degrades over time both

in vivo and *in vitro* depending on chemical, thermal and biological factors (Maniglia-Ferreira et al. 2007).

Biocompatibility is an important feature of all root canal filling materials considering that they may come in contact with the bone and periodontal ligament cells if extruded into the periapical tissues. MTT assay was used to assess the indirect cytotoxicity of the composite fibres using HOB cells *in vitro*. The eluants showed no statistically significant differences between any of the eluant-exposure scenarios and their respective negative controls regardless of eluant concentration. Preliminary cell proliferation studies using the Alamar Blue assay showed that the presence of hydroxyapatite did enhance proliferation in the long term; however, more studies are required to confirm the effect. Hydroxyapatite is established as a bone graft material, and it is most likely the explanation for this observation. Further studies are needed to evaluate the antibacterial properties of the silanated HA/PE composites, and micro-leakage studies and extensive tests to assess the sterilizability of the new materials are also needed.

Conclusion

The silanated HA/PE composites, LDPESHASR20/30 in particular, were developed as new root canal filling materials. The results of these investigations clearly demonstrate that the silanated composite tested is in general less susceptible to expansion and degradation in water and eugenol than gutta-percha; this might ensure a better seal in a filled root canal, particularly in the long term.

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References

- ANSI/ADA (2000) *Specification number 57 endodontic sealing material*. Chicago, USA: ADA Publishing.
- ASTM International (2002) *Standard test methods for vulcanized rubber and thermoplastic elastomers-tension*. West Conshohocken, PA: ASTM International. ASTM D412-98a (2002)e1.

- Bishop D, Griggs J, He J (2008) Effect of dynamic loading on the integrity of the interface between root canal and obturation materials. *Journal of Endodontics* **34**, 470–3.
- Bonfield W (1988) Composites for bone replacement. *Journal of Biomedical Engineering* **10**, 522–6.
- Camilleri J, Montesin FE, Di Silvio L, Pitt Ford TR (2005) The chemical constitution and biocompatibility of accelerated portland cement for endodontic use. *International Endodontic Journal* **38**, 834–42.
- Camps J, Pommel L, Bukiet F, About I (2004) Influence of the powder/liquid ratio on the properties of zinc oxide-eugenol-based root canal sealers. *Dental Materials* **20**, 915–23.
- Chandrasekhar V, Morishetty PK, Metla SL, Raju RV (2011) Expansion of gutta-percha in contact with various concentrations of zinc oxide-eugenol sealer: a three-dimensional volumetric study. *Journal of Endodontics* **37**, 697–700.
- Collins J, Walker MP, Kulid J, Lee C (2006) A comparison of three gutta-percha obturation techniques to replicate canal irregularities. *Journal of Endodontics* **32**, 762–5.
- Combe EC, Cohen BD, Cummings K (2001) Alpha- and beta-forms of gutta-percha in products for root canal filling. *International Endodontic Journal* **34**, 447–51.
- Deb S, Wang M, Tanner KE, Bonfield W (1996) Hydroxyapatite-polyethylene composites: effect of grafting and surface treatment of hydroxyapatite. *Journal of Materials Science: Materials in Medicine* **7**, 191–3.
- Guida A, Towler MR, Wall JG, Hill RG, Eramo S (2003) Preliminary work on the antibacterial effect of strontium in glass ionomer cements. *Journal of Materials Science Letters* **22**, 1401–3.
- Gutmann J, Witherspoon D (2002) Obturation of the cleaned and shaped root canal system. In: Cohen S, Burns R, eds. *Pathways of the pulp*, 8th edn. St Louis, MO: CV Mosby, pp. 293–364.
- Ikram OH, Patel S, Sauro S, Mannocci F (2009) Micro-computed tomography of tooth tissue volume changes following endodontic procedures and post space preparation. *International Endodontic Journal* **42**, 1071–6.
- Labella R, Braden M, Deb S (1994) Novel hydroxyapatite-based dental composites. *Biomaterials* **15**, 1197–200.
- Madison S, Swanson K, Chiles SA (1987) An evaluation of coronal microleakage in endodontically treated teeth. Part II. Sealer types. *Journal of Endodontics* **13**, 109–12.
- Maniglia-Ferreira C, Silva JB Jr, de Paula RC et al. (2007) Degradation of trans-polyisoprene over time following the analysis of root fillings removed during conventional retreatment. *International Endodontic Journal* **40**, 25–30.
- Michaud RA, Burgess J, Barfield RD, Cakir D, McNeal SE, Eleazer PD (2008) Volumetric expansion of gutta-percha in contact with eugenol. *Journal of Endodontics* **34**, 1528–32.
- Natera M, Pileggi R, Nair U (2011) A comparison of two gutta-percha obturation techniques to replicate canal irregularities in a split-tooth model. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontology* **112**, e29–34.
- Ng YL, Mann V, Gulabivala K (2011) A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. *International Endodontic Journal* **44**, 583–609.
- Nguyen NT (1994) Obturation of the root canal system. In: Cohen S, Burns RC, eds. *Pathways of the pulp*, 6th edn. St Louis: Mosby, pp. 219–71.
- Prosser IJ, Wilson AD (1982) Zinc oxide eugenol cements. VI. Effect of zinc oxide type on the setting reactions. *Journal of Biomedical Materials Research* **16**, 585–98.
- Santhoshkumar A, Palanivelu K, Sharma S, Nayak S (2010) A new synthesis of nickel 12-hydroxy oleate formulation to improve polyolefin's degradation. *Journal of Bioremediation & Biodegradation* **1**, 1–9.
- Scotti R, Tiozzo R, Parisi C, Croce MA, Bakdissara P (2008) Biocompatibility of various root canal filling materials ex vivo. *International Endodontic Journal* **41**, 651–7.
- Shipper G, Orstavik D, Teixeira FB, Trope M (2004) An evaluation of microbial leakage in roots filled with a thermoplastic synthetic polymer-based root canal filling material (resilon). *Journal of Endodontics* **30**, 342–7.
- Shipper G, Teixeira FB, Arnold RR, Trope M (2005) Periapical inflammation after coronal microbial inoculation of dog roots filled with gutta-percha or resilon. *Journal of Endodontics* **31**, 91–6.
- Stahlholz A, Friedman S, Heling I, Sela MN (1987) Efficiency of different chemical agents in decontamination of gutta-percha cones. *International Endodontic Journal* **20**, 211–6.
- Swanson K, Madison S (1987) An evaluation of coronal microleakage in endodontically treated teeth. Part I. Time periods. *Journal of Endodontics* **13**, 56–9.
- Tagger M, Greenberg B, Sela G (2003) Interaction between sealers and gutta-percha cones. *Journal of Endodontics* **29**, 835–7.
- Tay FR, Pashley DH, Williams MC (2005) Susceptibility of a polycaprolactone-based root canal filling material to degradation. I. Alkaline hydrolysis. *Journal of Endodontics* **31**, 593–8.
- Teixeira FB, Teixeira ECN, Thompson JY, Trope M (2004) Fracture resistance of roots endodontically treated with a new resin filling material. *Journal of the American Dental Association* **135**, 646–52.
- Tunga U, Bodrumlu E (2006) Assessment of the sealing ability of a new root canal obturation material. *Journal of Endodontics* **32**, 876–8.
- Weine F (1996) *Endodontic therapy*, 5th edn. St Louis: Mosby.
- Williams C, Loushine RJ, Weller RN, Pashley DH, Tay FR (2006) A comparison of cohesive strength and stiffness of resilon and gutta-percha. *Journal of Endodontics* **32**, 553–5.

Research Article

An In Vitro Assessment of Gutta-Percha Coating of New Carrier-Based Root Canal Fillings

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The first aim of this paper was to evaluate the push-out bond strength of the gutta-percha coating of Therafil and GuttaCore and compare it with that of gutta-percha used to coat an experimental hydroxyapatite/polyethylene (HA/PE) obturator. The second aim was to assess the thickness of gutta-percha around the carriers of GuttaCore and HA/PE obturators using microcomputed tomography (μ CT). Ten (size 30) 1 mm thick samples of each group (Therafil, GuttaCore, and HA/PE) were prepared. An orthodontic wire with a diameter of 0.5 mm was attached to the plunger of an Instron machine in order to allow the push-out testing of the gutta-percha coating. Five samples of (GuttaCore and HA/PE) were scanned using μ CT. The data obtained were analysed with one-way analysis of variance and Tukey post hoc test. HA/PE obturators exhibited significantly higher push-out bond strength ($P < 0.001$) determined at 6.84 ± 0.96 than those of GuttaCore around 3.75 ± 0.75 and Therafil at 1.5 ± 0.63 . GuttaCore demonstrated significantly higher bond strength than Therafil ($P < 0.001$). μ CT imaging revealed that the thickness of gutta-percha around the experimental HA/PE carrier was homogeneously distributed. The bondability and thickness of gutta-percha coating around HA/PE carriers were superior to those of GuttaCore and Therafil obturators.

1. Introduction

It is generally accepted that the outcome of the root canal treatment is positively correlated with the technical quality of the root canal obturation [1]. Previous studies have shown the effectiveness of thermoplasticized core carrier techniques in obtaining three-dimensional root canal fillings in a reduced amount of time compared to the lateral condensation technique [2–4]. Carriers for core-based techniques can be fabricated using different materials; Therafil small size obturators (up to size 40) (Tulsa Dental Dentsply, Tulsa, OK, USA) are made of Vectra, which is a liquid crystal polymer and larger sizes are made of polysulfone, whereas GuttaCore carriers (Tulsa Dental Dentsply) are made of cross-linked

gutta-percha. These materials are coated with alpha-phase gutta-percha.

The experimental carrier made of hydroxyapatite-polyethylene-strontium oxide was recently developed [5]. Most root canal filling materials do not thoroughly obturate the root canal system, leaving some voids either within the root filling material or at their interface with dentine [6]. These voids might harbour bacteria that can multiply when in contact with nutrients via the periapical region or lateral canals [7].

One potential disadvantage of a carrier-based root filling system is denudation of the core with stripping of the gutta-percha coating [8]. Stripping of gutta-percha from the carrier might happen during the insertion of the carriers into the

root canal space, particularly in narrow or severely curved canals. This would result in voids and inadequate filling of the root canal space [9]. Previous studies have shown that the most common causes of stripping of the gutta-percha coating are twisting the carrier during insertion into the root canal space and inadequate amounts of sealer placed prior to insertion of the obturators in the root canal [10, 11]. Adhesion between the carrier and gutta-percha coating is therefore an important aspect in the choice of a core-based obturation system and would help avoid stripping of the gutta-percha coating, creating a root canal filling with fewer voids.

Another potential disadvantage of currently available carrier-based obturation systems is that the volume of gutta-percha is not uniformly distributed around the carrier. This might cause stripping of the gutta-percha from the carrier material when the obturator is inserted into the root canal space leading to possible voids [8]. The frictional forces present between the gutta-percha and the root canal walls may create an extrusion effect, whereby the filling material is retained at the orifice of the canal [12]. High-resolution micro-CT is an emerging technology with several promising applications in many different fields of dentistry [13] including endodontics [14, 15]. Previous studies using the μ CT have shown the possibility of conducting both volumetric measurements [15] and 3D reconstruction of obturated root canals and their constituents [13].

To date, there have been no studies which have evaluated the strength of the bond between the carrier material and gutta-percha and the volume of gutta-percha around the carrier using μ CT. The purposes of the present study were therefore to compare the push-out bond strength between gutta-percha coatings and three types of carrier materials, in particular, Thermafil (Dentsply Tulsa Dental, Tulsa, OK), GuttaCore (Dentsply Tulsa Dental), and an experimental carrier made of hydroxyapatite/polyethylene (HA/PE) and strontium oxide, and to compare the thickness of gutta-percha between GuttaCore and HA/PE systems. The following hypotheses were tested: (i) the adhesion (push-out bond strength) between gutta-percha coating and the test carrier materials is the same and (ii) the thickness of gutta-percha coating around the experimental HA/PE carrier is not different from that of GC carrier.

2. Materials and Methods

2.1. Push-Out Test Specimen Preparation. The procedure used to fabricate the newly designed carrier has been previously described [5]. In brief, the hydroxyapatite (HA) powder with a mean particle size of 3–5 μ m (Plasma Biotol, Derbyshire, UK) was treated with a silane coupling agent solution (A174, Merck KGaA, Frankfurt, Germany) in order to improve the bond strength between the HA particles and the matrix, creating a more stable and durable composite material. Then the silanated HA (20 wt%) was mixed with (70 wt%) low-density polyethylene (Good Fellow Chemical Products, Huntingdon, England) and SrO (10 wt%) as a radiopacifier (Alfa Aesar, Ward Hill, UK) thoroughly and placed in a hopper of a 12 mm single screw extruder. The processing

temperature was maintained at 160°C and the speed of the rotary screw was optimized at around 25 rpm. The material was extruded through a die drawn down to different diameters and tapers. Subsequently, the composite carriers were successfully coated with gutta-percha (GP) to form the experimental carrier-based root canal obturator (HA/PE). This process was performed by dissolving the alpha phase of GP in chloroform until a viscous liquid was obtained. A cylindrical rubber tube with a stopper was prepared to receive the composite carrier material. GP was injected using a very fine needle into the tube, creating an evenly distributed layer of GP around the carrier [5].

Three types of endodontic obturators were tested: Thermafil (Dentsply Tulsa, OK), GuttaCore (Dentsply Tulsa), and the experimental hydroxyapatite/polyethylene (HA/PE) obturator as shown in Table 1.

Ten sized 30/.04 with 25 mm carriers from each carrier type were selected. The portion of each carrier coated with gutta-percha was divided into three parts, 5 mm long (coronal, middle, and apical). One slice (1 mm thick) was obtained from the junction point of the coronal portion and middle portion of each carrier using a low speed diamond wafering blade (Isomet; Buehler, Lake Bluff, IL) as shown in Figure 1(a). The diameters of the carriers in the portion used to obtain the slices were measured using a digital calliper (Maplin Electronics, Rotherham, UK) and were found to be 0.66 mm \pm 0.04 mm in case of GuttaCore and Thermafil obturators and 0.60 \pm 0.02 mm for the experimental HA/PE carriers. Each 1 mm thick slice was fixed to the aperture of a plastic syringe (Terumo, Leuven, Belgium) whose diameter was 1.5 mm in a vertical position using a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, USA) as shown in Figures 1(b) and 1(c). A 0.5 mm round orthodontic wire was attached to the plunger of a universal testing machine (Instron model 5569 A, High Wycombe, UK) in order to load the carrier at a constant crosshead speed of 0.5 mm/min (Figure 1(d)). A silicon-based material (Metrodent, Huddersfield, UK) was used to fix the syringe with its long axis parallel to the long axis of the plunger. On the loading machine, each slice was positioned with the larger side of the carrier segment placed facing the punch tip. Bond failure was considered to be the displacement of the carrier segment from the gutta-percha. Push-out strength data were determined in MPa by dividing the load in Newton by the bonded surface area (SL) in mm². SL was calculated using formula [16]: $SL = (R_1 + R_2) \sqrt{(R_1 + R_2)^2 + h^2}$, where $\pi = 3.14$, (R_1) is the apical carrier radius (base), (R_2) is the coronal carrier radius (top), and h is the height of the slice.

2.2. Microcomputed Tomography (μ CT) Specimen Preparation. Five further samples of GuttaCore (Dentsply Tulsa) and HA/PE (experimental) obturators were prepared from the middle third of the obturators (Figures 2(a) and 2(b)). These obturators were scanned using a GE Locus SP μ CT scanner (General Electric, London, ON, Canada) with an X-ray tube voltage of 80 kV and a current of 80 μ A. A 0.1 mm aluminium filter was used to attenuate the X-ray source. The specimens were immobilised using cotton gauze and

TABLE 1: Endodontic obturators used in this study.

Product	Manufacturer	Material
GuttaCore#30	Dentsply/Tulsa	Cross-linked gutta-percha
Thermafil#30	Dentsply/Tulsa	Vectra
HA/PE	Experimental	Hydroxyapatite-polyethylene-strontium oxide

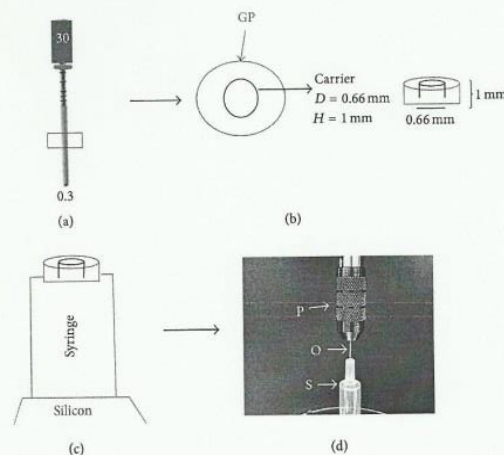


FIGURE 1: Schematic representation of the push-out test procedure. (a) The middle part of the endodontic obturator is chosen for the test. (b) Slices (height 1 mm and diameter 0.66 mm) are obtained. (c) The slice is fitted into the aperture of a customized syringe. (d) The plunger of the Instron machine is equipped with an orthodontic wire (0.5 mm in diameter) and aligned with the slice to be tested (P = Instron plunger, O = orthodontic wire, and S = syringe holding the slice).

scanned to produce $6.5 \times 6.5 \times 6.5 \mu\text{m}$ voxel size volumes. The samples were characterised further by making three-dimensional reconstructions of all the obturators. All scans were imported by Scan IP (Simpleware, Exeter, UK) as a stack of images where a segmentation had been carried out on each individual slice according to the pixel density. Two masks were produced to represent the gutta-percha coating and the carriers. The thickness of GP around its carrier was calculated along nine slices of each sample. Four identical fixed points on each slice were identified for thickness measured in both samples in order to make a comparison between GP-coated GC and HAPE cores as shown in Figures 2(c), 2(d), 2(e), and 2(f). Statistical analysis was performed using GraphPad Prism software version 5 (La Jolla, CA USA). One-way analysis of variance and Tukey post hoc test were used to determine any differences between the groups. The level of significance was set at $P = 0.05$.

3. Results

The findings of the push-out strength (MPa) are summarized in Table 2. The mean push-out bond strength of the experimental obturators, GuttaCore and Thermafil obturators, was

TABLE 2: Push-out bond strength of Thermafil, GuttaCore, and experimental carrier with gutta-percha coating. The data shows a statistically significant difference ($P < 0.001$) for the three different groups.

Product	Push-out bond strength in MPa
Thermafil	1.5 ± 0.63
GuttaCore	3.75 ± 0.75
Experimental HA/PE	6.84 ± 0.96

6.84 ± 0.96 , 3.75 ± 0.75 , and 1.5 ± 0.63 MPa, respectively. The push-out bond strength of the experimental carriers was found to be significantly higher ($P < 0.001$) than those of GuttaCore and Thermafil. GuttaCore push-out bond strength was significantly higher than Thermafil ($P < 0.001$).

The μCT scans demonstrated that the volume of gutta-percha in the HA/PE system was more symmetrical and homogenous than that of GuttaCore as shown in Figure 2. The thickness of gutta-percha in the GuttaCore system from four points "3, 6, 9, 12" was 0.376 ± 0.027 , 0.164 ± 0.023 , 0.357 ± 0.033 , and 0.591 ± 0.034 mm, respectively. On the other hand, the HA/PE showed a thickness of gutta-percha

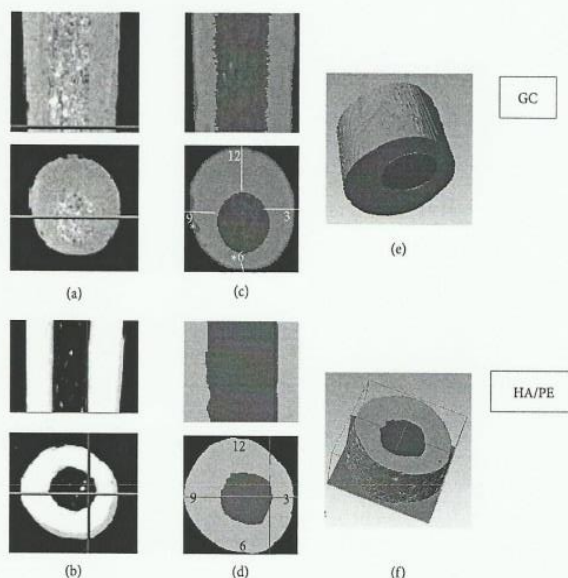


FIGURE 2: Microcomputed tomography images prepared from the middle third of the GC and HA/PE obturators. (a, b) μ CT scans of the GC and HA/PE systems, respectively, showing slice selection from the middle third. (c, d) μ CT slices of GC and HA/PE, respectively, segmented by Scan IP using different masks. (e, f) 3D reconstruction of GC and HA/PE systems using Scan IP based on voxel density.

coating at "3, 6, 9, 12" o'clock positions to be 0.419 ± 0.03 , 0.607 ± 0.042 , 0.584 ± 0.039 , and 0.535 ± 0.04 mm, respectively. The difference in volume of gutta-percha between GuttaCore system and HA/PE was significant ($P < 0.05$) particularly at the "6" and "9" o'clock positions.

4. Discussion

Carrier-based techniques are prone to exposure to the carriers during their insertion into the root canal, which would cause the formation of voids between the root filling material and the root canal walls. Adhesion between the gutta-percha and carrier material is therefore an important requirement to effectively obturate the root canal system. Stripping of gutta-percha from the carrier has been observed by previous researchers in the apical third and in the middle and apical thirds [17, 18]. We developed carriers of different diameters and tapers made of hydroxyapatite-polyethylene (HA/PE) fibres, which could be easily removed should retreatment be necessary and with micromechanical adhesion between the gutta-percha and carrier. This newly designed carrier has been described in a previous paper [5].

To the authors' knowledge this is the first paper in which a push-out test has been used to test the bond strength between the GP coating and the carrier of core-based root canal filling systems. Although the expected push-out values that would

prevent gutta-percha stripping off in a carrier-based obturator during the obturation procedure are still unknown, the satisfactory adhesion of gutta-percha to the carrier material may be effective. It is worthwhile clarifying two points regarding the geometry of the carrier and their possible effects on the push-out results. The first issue is related to the presence of a groove in Thermanfil and GuttaCore carriers. This groove has been devised to facilitate the retrieval of the carriers by use of a hedstrom file in case of retreatment. The groove is wider in the coronal part than in the middle part of the carrier, and the slices to be subjected to the push-out test were therefore prepared from the middle part of the obturator in order to reduce to a minimum the surface alteration caused by the presence of the groove. In addition, the taper of the carrier in the tested endodontic obturators was 0.04 mm; this taper would not have excessively influenced the results, considering that the tested slices of the carriers were only 1 mm thick. Moreover, the push-out bond strength values were calculated according to the equation estimating the apical taper radius and coronal taper radius to obtain accurate results. The bond between the gutta-percha and the carrier of the experimental obturator was significantly higher than the one obtained with Thermanfil and GuttaCore. This could be due to the micromechanical adhesion of the GP to the HA/PE fibres as the fibre-based carriers have irregular surfaces. Similarly, the push-out bond strength of the GuttaCore carrier was

found to be significantly higher than that of the Thermafil carrier. Again the GuttaCore carrier appeared to offer better micromechanical retention than the Thermafil carrier, since the material used to fabricate the GuttaCore carrier is cross-linked GP and so it is unlikely that any chemical interaction would develop between this and the alpha-phase GP of the coating. The clinical relevance of the information we gathered on the push-out bond strength of the gutta-percha to the carriers is, however, somehow limited, considering that in a clinical context the carriers are heated before insertion into the root canal and this might result in the increased bond strength of the gutta-percha to the carrier.

The present research used a model that allowed for the assessment of the thickness of gutta-percha around the carrier material outside the root canal system using μ CT technology. Thermafil and GuttaCore carriers are identical in shape and the gutta-percha used for the coating is also identical for the two systems; it was for this reason that only one of the two carrier types (GuttaCore) was used for the measurement of the thickness of the gutta-percha. μ CT technology has been used to assess root canal morphology [19, 20] and root canal obturation [15], quantitatively as well as qualitatively. Also, μ CT analysis has been used to evaluate root canal fillings and other dental materials in 3D reconstructions [21, 22]. In this study the μ CT scanner accurately quantified the distribution of gutta-percha around the carriers. In the GuttaCore carrier the thickness of GP was not uniformly distributed around the carrier and the carrier seemed to be positioned eccentrically. On the other hand, μ CT imaging showed that the thickness of GP on the HA/PE carrier was rather evenly distributed and the carrier was in the centre of GP. Further studies are required in order to investigate the push-out bond strength and the quality of the root canal fillings obtained using the most recently developed core-based systems particularly in curved canals. The present study concludes that the experimental HA/PE carrier exhibited the highest push-out bond strength and the new GuttaCore carriers showed a significant improvement in push-out strength compared with the Thermafil ones; thus the first hypothesis has been rejected. The second hypothesis has also been rejected because the thickness of gutta-percha coating of HA/PE was uniform in comparison to that of GuttaCore, which was not homogenous. It is expected that a good retention of gutta-percha around the carrier with a uniform thickness of gutta-percha coating would help prevent stripping of gutta-percha coating around the carrier material.

Conflict of Interests

The authors declare no conflict of interests.

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References

- [1] Y.-L. Ng, V. Mann, S. Rahbaran, J. Lewsey, and K. Gulabivala, "Outcome of primary root canal treatment: systematic review of the literature—part 2. Influence of clinical factors," *International Endodontic Journal*, vol. 41, no. 1, pp. 6–31, 2008.
- [2] T. A. Becker and J. C. Donnelly, "Thermafil obturation: a literature review," *General Dentistry*, vol. 45, no. 1, pp. 46–5560, 1997.
- [3] M. Lipski, "Studies comparing the efficacy of root canal filling with Gutta-Percha lateral condensation and Thermafil obturators," *Annales Academiae Medicae Stetinensis*, vol. 46, pp. 317–330, 2000.
- [4] K. Clinton and T. van Himel, "Comparison of a warm Gutta-Percha obturation technique and lateral condensation," *Journal of Endodontics*, vol. 27, no. 11, pp. 692–695, 2001.
- [5] R. A. Alhashimi, F. Mannocci, R. M. Foxton, and S. Deb, "Synthesis and preliminary evaluation of a polyolefin-based core for carrier-based root canal obturation," *Journal of Endodontics*, vol. 38, no. 7, pp. 983–986, 2012.
- [6] E. G. Kontakiotis, G. N. Tzanetakis, and A. L. Loizides, "A 12-month longitudinal in vitro leakage study on a new silicon-based root canal filling material (Gutta-Flow)," *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology*, vol. 103, no. 6, pp. 854–859, 2007.
- [7] L. W. M. van der Sluis, M.-K. Wu, and P. R. Wesselink, "An evaluation of the quality of root fillings in mandibular incisors and maxillary and mandibular canines using different methodologies," *Journal of Dentistry*, vol. 33, no. 8, pp. 683–688, 2005.
- [8] E. Rapisarda, A. Bonaccorso, and T. R. Tripi, "Evaluation of two root canal preparation and obturation methods: the Mc Spadden method and the use of ProFile-Thermafil," *Minerva Stomatologica*, vol. 48, no. 1-2, pp. 29–38, 1999.
- [9] R. N. Weller, W. F. Kimbrough, and R. W. Anderson, "A comparison of thermoplastic obturation techniques: adaptation to the canal walls," *Journal of Endodontics*, vol. 23, no. 11, pp. 703–706, 1997.
- [10] M. E. Levitan, V. T. Himel, and J. B. Luckey, "The effect of insertion rates on fill length and adaptation of a thermoplasticized Gutta-Percha technique," *Journal of Endodontics*, vol. 29, no. 8, pp. 505–508, 2003.
- [11] K. A. DuLac, C. J. Nielsen, T. J. Tomazic, P. J. Ferrillo Jr., and J. E. Hatton, "Comparison of the obturation of lateral canals by six techniques," *Journal of Endodontics*, vol. 25, no. 5, pp. 376–380, 1999.
- [12] A. Bertacci, C. Baroni, L. Breschi, M. Venturi, and C. Prati, "The influence of smear layer in lateral channels filling," *Clinical Oral Investigations*, vol. 11, no. 4, pp. 353–359, 2007.
- [13] M. Jung, D. Lommel, and J. Klimek, "The imaging of root canal obturation using micro-CT," *International Endodontic Journal*, vol. 38, no. 9, pp. 617–626, 2005.
- [14] F. B. Barletta, N. D. M. Rahde, O. Limongi, A. A. M. Moura, C. Zanesco, and G. Mazocatto, "In vitro comparative analysis of 2 mechanical techniques for removing Gutta-Percha during retreatment," *Journal of the Canadian Dental Association*, vol. 73, no. 1, article 65, 2007.

- [15] M. Hammad, A. Qualtrough, and N. Silikas, "Three-dimensional evaluation of effectiveness of hand and rotary instrumentation for retreatment of canals filled with different materials," *Journal of Endodontics*, vol. 34, no. 11, pp. 1370–1373, 2008.
- [16] L. Giachetti, D. S. Russo, M. Baldini, F. Bertini, L. Steier, and M. Ferrari, "Push-out strength of translucent fibre posts cemented using a dual-curing technique or a light-curing self-adhering material," *International Endodontic Journal*, vol. 45, no. 3, pp. 249–256, 2012.
- [17] D. S. Clark and M. E. ElDeeb, "Apical sealing ability of metal versus plastic carrier thermafil obturators," *Journal of Endodontics*, vol. 19, no. 1, pp. 4–9, 1993.
- [18] V. Kytridou, J. L. Gutmann, and M. H. Nunn, "Adaptation and scalability of two contemporary obturation techniques in the absence of the dentinal smear layer," *International Endodontic Journal*, vol. 32, no. 6, pp. 464–474, 1999.
- [19] R. B. Nielsen, A. M. Alyassin, D. D. Peters, D. L. Carnes, and J. Lancaster, "Microcomputed tomography: an advanced system for detailed endodontic research," *Journal of Endodontics*, vol. 21, no. 11, pp. 561–568, 1995.
- [20] S. E. P. Dowker, G. R. Davis, and J. C. Elliott, "X-ray microtomography: nondestructive three-dimensional imaging for in vitro endodontic studies," *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, vol. 83, no. 4, pp. 510–516, 1997.
- [21] M. Hammad, A. Qualtrough, and N. Silikas, "Evaluation of root canal obturation: a three-dimensional in vitro study," *Journal of Endodontics*, vol. 35, no. 4, pp. 541–544, 2009.
- [22] M. M. Hatamleh and D. C. Watts, "Porosity and color of maxillofacial silicone elastomer," *Journal of Prosthodontics*, vol. 20, no. 1, pp. 60–66, 2011.